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PYRAZINE RETENTION DURING PRODUCTION OF DEHYDRATED POTATO GRANULES

by

© PATRICK D. MARCE

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF

MASTER OF SCIENCE

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EDMONTON, ALBERTA

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THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled

"PYRAZINE RETENTION DURING PRODUCTION OF DEHYDRATED POTATO GRANULES"

submitted by PATRICK D. MARCE in partial fulfilment of the requirements for the degree of Master of Science.

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May 24, 1977

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ABSTRACT

The levels of natural pyrazines in granules from an add-back process and in potato flakes, and pyrazine retention during a freeze-thaw granule process, were monitored. Prior to this, the retention of pyrazines was studied in model systems of dichloromethane eluted columns packed with pure potato cellular constituents, including starch, amylose, amylopectin, pectin, cellulose and protein, as well as add-back dehydrated granules. Alkylated pyrazines and 2-methoxy-3-ethyl-pyrazine were used in both retention studies. When incorporated into moist mash, which was then subjected to dehydration steps, the losses of pyrazines were 65.7-85% in the pre-drying step, with additional losses of 14.3-18.3% in the granulation step, while final drying brought about a granule end-product in which the loss was 99.9%. The extent of pyrazine losses appeared to be dependent only on the amount of moisture removed. Retention of pyrazines by pure potato component matrices and add-back granules was generally very low, with most values falling in the range of 0.1-25% relative retention. Of the seven matrices studied, microcrystalline cellulose showed the highest pyrazine relative retention value, with an average of 32.7% after collection of 20 fractions, and potato protein gave the next highest value of 17.2%, while the other components, including the add-back granules, displayed very low retaining abilities. Qualitative and quantitative analyses were based on the application of differential pulse polarography instead of conventional gas liquid chromatography. Half-wave potentials of pyrazines in acetate buffer, pH 4.90, were reported, and an extraction procedure, which improved the detection limit of pyrazines to a level of 1 part per 10^9, was developed.

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I. INTRODUCTION

During recent years, a marked shift in consumption trends has occurred within potato markets. Changes in potato utilization have shown substantial increases in the consumption of processed potato products as opposed to that of fresh table potatoes. Thus, french fried potatoes, potato chips and instant dehydrated mashed potatoes (either granular or flaked) have gained notable popularity. The popularity of dehydrated potato granules, as a convenience food, is widespread. Increasing demand domestically and, particularly, abroad has resulted in a steady increase in production which, in the past year, was 30% in Alberta alone.

Although potatoes and potato products are nutritious and constitute a significant portion of our diet, they must, to be accepted, possess a high organoleptic quality, of which flavor is a leading factor. From a consumer standpoint, flavor ranks as one of the most important properties of instant mashed potatoes, as with any other processed foodstuff. The bland flavor of dehydrated granules has necessitated, among other things, a great deal more knowledge concerning the behavior of natural flavoring constituents during processing. In previous studies related to potato flavor, the bulk of reported findings were concerned only with qualitative aspects involved in the flavor of raw, cooked or boiled, and baked potatoes, as well as potato chips, granules and flakes. Very little attention has been paid to quantitative evaluations of potato odor and, in particular, a lack of information exists pertaining to the contents of this essence during the processing of the dehydrated product.

Hence, the aim of this study was to determine the level of pyrazines - major potato flavor constituents - in granules of the add-back process, and in potato flakes, and monitor their retention during a freeze-thaw granule process. In addition, in an attempt to explain the behavior of these flavor compounds on a molecular basis, a further objective was to assess pyrazine retention in model systems of the following pure potato cell matrix components: starch, amylose, amylopectin, pectin, cellulose and protein. The data might then suggest modifications in the process to ensure and maintain flavor improved end-products.

II. REVIEW OF THE LITERATURE

A. Potato flavor

In normal cooked potatoes, none of the primary taste sensations of a salty, sour, sweet or bitter nature are ordinarily perceptible. The only evidence of any basic taste properties has been found in improperly stored potatoes, which may become sweet, and in potatoes exposed to light for long periods of time, which may turn bitter. In addition to taste, the term "flavor", when applied to potatoes, is also commonly used to denote a whole complex of sensations including aroma, mouth-feel, texture, and even appearance (Burr, 1966). While flavor of potatoes and potato products has been studied for some time (Ryder, 1966; Self, 1967), an interpretation of the significance of the findings has been difficult. Investigations involving potato flavor have ranged from work on the aroma of raw potatoes to a variety of studies with baked, and cooked or boiled potatoes, as well as potato chips. Results arising from these flavor studies show that a very complex spectrum of constituents is responsible for the occurrence of a potato flavor.

1. Non-volatile constituents

The relationship between flavor and non-volatile constituents of potatoes has been studied. Buri *et al.* (1970) stressed the importance of free amino acids and 5'-nucleotides for the basic flavor note; however, they did not present a mechanism for the formation of 5'-nucleotides during cooking. Although 5'-nucleotides play important roles as flavor potentiators, they possess no taste of their own unless present in or added to potatoes or other foods (Dumelin and Solms, 1976). When free amino acids, particularly glutamic acid, are present con-

currently with the nucleotides, the flavor enhancement effect is synergistically increased (Solms, 1971).

The nucleotide content of raw potatoes, though low, is comparable to that of other starch containing plant materials. Small amounts of adenosine- and uridine-nucleotides are found in the raw tubers (Rees and Duncan, 1972; Duncan and Rees, 1972); however, upon cooking, relatively high levels of the 5'-nucleotides are formed. Dumelin and Solms (1976) showed that the method of formation of these compounds is probably due to a complex enzymatic hydrolysis of tuber RNA.

In general, the study of non-volatile potato flavorings has been marginal. Other non-volatiles, such as phenolics and glycoalkaloids, have been considered significant in potato flavor development, although the majority of these compounds were associated with an off-flavor type bitterness (Sinden and Deahl, 1976).

2. Volatile constituents

The essence of potato is comprised of a variety of functionally different compounds, the major classes being sulfur containing compounds, carbonyls, thiazoles and, most notably, pyrazines.

The volatile constituents of potatoes contributing to odor, especially those in the low boiling range, were identified and thoroughly discussed and reviewed by Self (1967), Burr (1966), and Johnson *et al.* (1971); however, none of these volatiles had a characteristic potato odor. Most of these earlier studies were concerned with components more volatile than hexanal.

More recent work, particularly by Buttery *et al.* (1970), has resulted in characterization of higher boiling components. The medium and high boiling range potato volatiles, obtained by vacuum distill-

ation, were 0.1 ppm in raw Russet Burbank potatoes, and had an aroma similar to that of raw potatoes. The major components were 1-octen-3-ol, trans-2-octenal, trans-2-octenol and geraniol. The volatile oil obtained at atmospheric pressure, in an amount equivalent to 1 ppm in the potato, had an aroma similar to that of cooked potatoes, and among the major components identified were 2-pentylfuran, phenylacetaldehyde, hexanal, trans-2-furfurol and pyridine. In the same report, tentative evidence was given for the presence of 2-methoxy-3-ethylpyrazine, now known to be a potent odorant in raw potatoes.

The pattern of a potato aroma profile is highly dependent upon the form of the potatoes, and extensive treatment has been given to the study of volatile constituents in raw, boiled, steam cooked, fried and baked potatoes, as well as potato chips.

a. Sulfur containing compounds

The importance of sulfur containing compounds to potato flavor lies in their extremely low odor thresholds, ranging in the order of 5 to 20 ppb in water for compounds such as hydrogen sulfide, methyl mercaptan and dimethyl mono- and disulfides (Gumbmann and Burr, 1964). The same authors, in an investigation of the volatile sulfur compounds from cooked potatoes, identified fourteen sulfur compounds, with methyl mercaptan and methyl sulfide making up 90% of the total mixture. They further stated that it may, on first thought, seem anomalous that such an array of highly odoriferous compounds occur in a food as bland as potatoes. This was clarified by the explanation that sulfur compounds are important in providing only secondary flavoring characteristics in cooked potatoes, since it was felt that these compounds, at extremely low concentrations, lose their characteristic odor.

The most notable sulfur containing constituent of potato aroma is 3-methylmercaptopropanal (methional), its importance in the nonbasic volatile fraction being established by Buttery and Ling (1972), Guadagni *et al.* (1972) and Buttery (1973). This compound is thought to be one of the more important volatile constituents of baked potatoes (Buttery *et al.*, 1973), potato chips (Buttery and Ling, 1972) and cooked, and dehydrated, granular potatoes (Nursten and Sheen, 1974). Guadagni *et al.* (1971) conducted an experiment for the flavor enhancement of dehydrated mashed potatoes with methional, and found it to improve the flavor of one sample of dehydrated flakes. Finally, the formation of a potato chip-like flavor from the thermal oxidation of methionine, added to the frying oil, has been attributed to methional (Lee *et al.*, 1973).

b. Carbonyls

A multitude of carbonyl constituents in potato aroma have been identified and reported by various workers. Johnson *et al.* (1971), in a review of volatile compounds identified in potatoes, reported a total of 34 carbonyls - consisting of both aliphatic and aromatic aldehydes and ketones, including methional - which had been previously characterized; of these constituents, 24 were found in boiled potatoes and only 11 were present in fresh tubers.

Mookerjee *et al.* (1965) identified 18 monocarbonyls in fresh potato crisps, the major compounds being hexanal, pentanal, 2-pentanone, acetone, 2-heptenal and 2-octenal; however, they felt that the majority of these carbonyls probably originate from the fat used during cooking and not from the potato.

A number of aldehydes had been previously characterized

in potato chips (Buttery and Ling, 1972; Chang, 1967; Mookerjee *et al.*, 1965), but these were generally of the commonly encountered straight chain saturated, monounsaturated and diunsaturated types.

Some unusual aldehydes were detected in potato chips by Buttery (1971), and further to this, Buttery (1973) identified these uncommon volatile carbonyl compounds. He reported a total of 8 aldol condensation type compounds, as well as 4 other unusual carbonyls.

c. Thiazoles

As outlined by Buttery *et al.* (1971), most of the components from the basic fraction of the volatile oil of potato chips are alkylpyrazines. There were, however, a few components with an odd numbered molecular weight, indicating compounds containing only one nitrogen (or an odd number) in the molecule. Subsequent to this, both alkyl- and alkanoylthiazoles were found in potato chips (Buttery and Ling, 1974). 2,4,5-Trimethylthiazole and 2-isopropyl-4,5-dimethylthiazole were characterized in the basic fraction of the volatile oil of the chips. As well, these compounds, and the additional compounds of 2-isopropyl-4-methyl-5-ethylthiazole, 2-isobutyl-4,5-dimethylthiazole and 2-acetylthiazole, were characterized in the basic fraction of the volatile oil from boiled potatoes. These thiazoles occurred at about the same concentration as many of the minor alkylpyrazines, but were approximately one tenth of the amount of the major alkylpyrazines. It is thought that, due to their potent and characteristic odor, some of these compounds could be important to the aroma and flavor of potato products.

d. Pyrazines

Deck and Chang (1965) had reported 2,5-dimethylpyrazine,

which they isolated from potato chips, as possessing the odor of potatoes. Similar alkylpyrazines had also been reported earlier (Schorigin *et al.*, 1933). However, none of these compounds were detected by Buttery *et al.* (1970) as a result of their study of some volatile potato components.

More recent work has resulted in the characterization of numerous pyrazines in the volatile essence of raw, cooked, fried and baked potatoes, as well as potato chips. Buttery *et al.* (1971) found 18 pyrazine compounds in the basic fraction of the steam volatile oil from potato chips, 8 compounds of which had been previously reported as being present in potato chips by Chang and coworkers (Deck and Chang, 1965; Chang, 1967), and a number of which had been found by Sapers *et al.* (1971) in explosion puffed dehydrated potatoes. Preliminary sensory evaluation indicated that 2-ethyl-3,6-dimethylpyrazine is one of the most potent odorants derived from chips, and that, in low concentrations, it could make a definite contribution to potato chip aroma.

Pyrazines have recently been characterized in cooked potato (Nursten and Sheen, 1974) and baked potato (Buttery *et al.*,1973; Pareles and Chang, 1974). Though only traces of pyrazines were found in boiled potatoes, relatively large amounts were found among the volatiles of baked potatoes. The concentration of volatile oil constituents in baked potatoes was ten times less than that in potato chips, but the pattern of compounds found, with the exception of a "cracker odor" constituent, was similar to that of chips. A total of 42 compounds were found in baked potato odor, and among the compounds contributing the most to the aroma were 2-ethyl-3,6-dimethylpyrazine and 2-ethyl-3,5-dimethylpyrazine.

The earthy aroma of potatoes was assigned to the presence of 2-methoxy-3-isopropylpyrazine (Buttery and Ling, 1973). Its concentration in the tuber was in the order of 1 part in 10^{10}. In the same report, the authors also indicated the possible presence of 2-methoxy-3-ethylpyrazine, and suggested its concentration to be less than 1 part in 10^{12}. Guadagni *et al.* (1971), in a sensory study on the effect of adding both compounds separately to reconstituted dehydrated potatoes, found that, in contrast to the isopropyl derivative, only the ethyl pyrazine was able to effectively improve the flavor of the potatoes. In relation to this finding, Seifert *et al.* (1970) showed that the methoxy group, more than any other alkoxy group, is the major contributor to the flavor. For example, the odor threshold of 2-ethoxy-3-ethylpyrazine (11 ppb), when compared with that of 2-methoxy-3-ethylpyrazine (0.4 ppb), showed a decrease in potency by a factor of about 27 times.

The most conclusive evidence for the presence of 2-methoxy-3-ethylpyrazine in potatoes, and its relation to raw earthy odor, was given by Nursten and Sheen (1974). Based on a threshold value of 4.2 parts per 10^{10} in water, they calculated its concentration in cooked potatoes to be less than 10 parts per 10^{12}. They also found that dehydrated potato granules retained little of the raw potato character, with the yield of volatiles from granules being about five times lower than that from freshly prepared mashed potatoes. They suggested that the inferiority in flavor of dehydrated potatoes should be attributed to lack of volatiles rather than to a specific off-flavor developed during processing. Finally, Guadagni *et al.* (1971) gave evidence of the important role of 2-methoxy-3-ethylpyrazine in potato flavor by showing

that the flavor of several samples of dehydrated granules and flakes was consistently and significantly improved by the addition of this compound in amounts of not more than 0.1-0.2 parts per 10^6, while other volatiles had no such effect.

Thus, it is widely accepted among workers in the field of potato flavor chemistry that, of the volatile constituents found in potatoes, the pyrazines are the single most important group of compounds contributing to potato aroma.

3. Patents

The occurrence of pyrazine derivatives in natural products was indicated for the first time by Morin in 1888. Not before 1928, however, was it discovered that these types of compounds were useful aroma constituents. Then a patent was granted to Reichstein and Staudinger (1928) for the use of pyrazines in combination with other compounds used in the preparation of synthetic coffee oil. Since then a variety of patents have been issued for the incorporation of synthetic pyrazines into many food commodities, including potato products.

Several patents have been developed for flavor enhancement of processed potatoes with synthetic pyrazines (Flament, 1974; Guadagni *et al.*, 1973; Mookerjee *et al.*, 1973). The most notable of these patents is that of Guadagni *et al.* (1973), which suggests the addition of about 0.1-0.3 ppm of the hydrochloric acid salt of 2-methoxy-3-ethylpyrazine to potatoes and potato products. The patent of Mookerjee *et al.* (1973) recommends the use of a potato flavoring containing 0.005-20 ppm 2-acetyl-3-ethylpyrazine in a carrier. A 0.2% amount of this flavoring produced a potato flavor in a cracker-type snack product. Flament (1974) developed a patent in which combinations of pyrazines, cyclohexenones

and (or) thiazolidines were used to enrich the flavor of french fried potatoes. A patent for the synthesis of 2-methoxy-3-isobutylpyrazine was given to Buttery *et al.* (1973), however, the authors do not specify a recommendation for the addition of this compound to any particular foodstuff. One of the more often cited patents for the improvement of potato-like flavor is that of Chang (1970), in which he claims that methionine imparts a chip-like flavor to oil in which it is heated. The credibility of those patents concerning potato flavor enhancement, however, cannot be established at this time since their feasibility for implementation in potato processing was not well elaborated.

B. Interaction of flavor volatiles with foods and food components

The study of flavor compound binding in foods has not been extensive since whole, intact foodstuffs present difficult substrates for sorption experiments (the term binding is equated to the concept of sorption in the widest sense, including adsorption and absorption, as well as physical and chemical binding), because of their heterogeneous composition (Maier, 1972a). As a result, the majority of investigations concerning this flavor volatile behavior have dealt with interactions between the flavor compounds and model food components, including macromolecular constituents such as lipids, carbohydrates and proteins, as well as lower molecular weight compounds (for example, sucrose and sodium chloride).

The interaction of volatiles with model food components was thoroughly discussed and reviewed by Solms *et al.* (1973). The authors stated that there seems to be no universal mechanism for flavor binding in foods. They did conclude, however, that there are at least three major but much different mechanisms responsible for the interaction

of volatiles with food components. The first, and simplest, of these involves physical laws of partition, in which interactions can be characterized with the partition coefficients for individual compounds, while structural effects are not known to be of importance. Secondly, flavor volatiles may associate with food components if secondary and tertiary structures of the solid matrices are formed, which confer upon the molecules new binding zones that can then interact with the ligands. This reaction apparently requires some sort of activation. A third mechanism known to be responsible for flavor binding is a hydrophobic one, in which the food component interacts with ligands (flavor molecules) under unfolding and destruction of tertiary structures, liberating binding zones as a consequence, and thus, the reaction depends partially on the "unfolding capacity" of the flavor molecules for the food complex. The nature and extent of these major binding mechanisms and other less important ones is largely dependent, however, upon the nature of the food component under consideration.

1. Flavor binding in model food components

a. Lipids

The interaction of flavor volatiles with lipid materials is probably the most well-defined when compared to binding by other macromolecular constituents. The method for flavor binding in lipids appears to primarily follow the partition mechanism described previously. Lipids are rather homogeneous, hydrophobic and non-polar materials, existing in aqueous medium in the form of distinct regions. In these systems, flavor compounds will be distributed between the lipid and the aqueous phases, following the physical laws of partition, and giving, under ideal conditions, a true equilibrium. Meyer *et al.* (1920) deter-

mined the distribution of butanol and vanillin in the system olive oil-water and, as a result of this, it was shown that, in compounded foods with varying amounts of fat, the flavor dosage factor - that is, the flavor dose necessary to always obtain the same flavor sensation - increases approximately proportionally to the fat content (Van Eijk, 1971).

In addition to the partition effect in lipid-flavor interactions, the possibility of physical binding mediated by van der Waals' forces has been inferred by Maier (1972a). This suggestion was based on the infrared spectra of tripalmitin, isopentanol and isopentanol sorbed on tripalmitin.

b. Carbohydrates

The retention of volatiles in systems rich in carbohydrates is much more complex than for lipid compounds. Polysaccharides, particularly starch, are predominantly associated with flavor compounds in accordance with the second major binding mechanism mentioned earlier. Specifically, starch (especially the amylose fraction) can form helical structures (although these are severely retrograded in the case of pure amylose), inside which is an electron cloud of very high density that presumably interacts with the electron systems of the molecules entering these structures. Therefore, the flavor molecules, rather than chemically reacting with the macromolecules, are physically entrapped within the helical structures, and thus, the term inclusion complex formation has been used to describe this binding. In support of this, Maier (1972a) found that, in amylose and amylopectin complexed with phenol, guaiacol, vanillin and thymol, a substantial portion of the flavoring could not be removed with dry ether even after one year.

In addition to the formation of starch, amylose and amylopectin inclusion complexes, most workers agree that other possibilities exist for flavor binding in these components and other carbohydrates. Several investigators assume, for example, that flavor ligands are also bound to preferential binding sites on the exterior of the helix or to other regions of the macromolecules (Simpson, 1970; Nicolson *et al.*, 1966a,b). In this case, physical binding via hydrogen bonding would be the most likely interaction. Acid-base interactions are also possible between flavoring compounds and carbohydrate macromolecules possessing a net acidic or basic character. Acidic macromolecules such as pectin, pectic acid and alginic acid, for example, were shown to form salts with volatile amine compounds (Maier, 1972a).

c. Proteins

The hydrophobic mechanism of flavor binding seems to be the main phenomenon occurring between proteins and volatile flavor molecules (Solms *et al.*, 1973). Although the hydrophobic regions of the protein molecules are of importance for the stabilization of protein structure, they are also available for interactions with apolar organic molecules of low molecular weight, and there are mainly two effects which are typical for these flavor-protein interactions (Némethy, 1967): the solubility of the ligand (flavor) in the total system is increased due to adsorption at the protein surface or due to inclusion in the hydrophobic regions; and the ligand interacts with the hydrophobic zones of the protein and opens up these regions.

Evidence for binding of flavors by proteins has been presented recently by Arai *et al.* (1970) and Beyeler and Solms (1974). The former authors demonstrated that n-hexanal and n-hexanol were able

to firmly bind several aldehydes, ketones and alcohols (in the same report, they showed that both proteins had absolutely no affinity for 2,5-dimethylpyrazine, under the set of experimental conditions employed in the binding studies).

2. Volatile retention in food models during processing

Much work has been done on the retention or loss of volatiles under non-equilibrium conditions, namely during food processing operations, like evaporation and drying. Under these conditions, quite other effects, like mass transport at different dry matter concentrations, determine the reaction (Menting *et al.*, 1967, 1970). Generally, in processes of dehydration, loss of non-chemically bound volatile aromas is controlled by the value of their diffusion coefficients and is nearly independent of the relative volatility (Thijssen, 1972). The diffusion coefficients and, consequently, the loss by evaporation decrease with an increasing size of the aroma molecule. However, even small aroma molecules, such as the lower aldehydes, ketones, esters and alcohols, can be fully retained if the water concentration in the liquid at the evaporating surface can be dropped to a very low value right after the drying has started. Process conditions leading to a maximum aroma retention can be calculated theoretically. Those optimum conditions can be approached in spray-drying, slab-drying and freeze-drying. Although very little information exists regarding flavor retention during fluidized bed drying in particular, Thijssen claims that, for fully optimized drying conditions, the aroma retention and quality of air-dried foods can be equal to that for freeze-dried foods.

a. Spray-drying

In the process of spray-drying, where liquid is atomized

in a current of hot air, aromas are lost only during the time needed to lower the surface water concentration of the droplet to the critical value. Because of the high mass transfer coefficient in the gas phase around the small droplets, this critical water concentration is attained within a few milliseconds. Process conditions leading to a decrease in this time therefore result in an increase in flavor retention. Thijssen (1972), in a study of volatile retention by spray-dried malto dextrin, showed that very high retentions could be achieved even with extremely volatile aroma molecules like acetone.

While flavor volatile retention during spray-drying of various foods and food components has been extensively studied by various workers, the interpretation of the results has been concerned primarily with physical laws (such as mass transfer phenomena) as totally governing flavor retention, with no reference to flavor-matrix interactions on a molecular basis (for example, chemical and electrostatic forces).

b. Slab-drying

In this dehydration procedure, heat is supplied to the food or food component through direct contact with a heated surface (drum or belt). According to Thijssen (1972), flavor retention is again dependent on the critical moisture level, however, mass transfer effects are not considered as important to volatile retention as the initial dissolved solids level (although flavor-solid interactions during slab-drying were not discussed).

c. Freeze-drying

Of the many available dehydration processes, freeze-drying is accepted as one of the better schemes for flavor retention. An important advantage is the low processing temperature, which excludes

thermal degradation and gives the product structural rigidity. It is believed that the retention of organic volatiles results from surface adsorption of the volatile on the dry layer of the freeze-drying sample (Rey and Bastien, 1962) or from an entrapment mechanism which immobilizes the volatile compounds within the amorphous solute matrix (Flink and Karel, 1970a; Thijssen, 1971; Thijssen and Rulkens, 1969; Chandrasekaran and King, 1971). The physical aspects by which the volatile is entrapped within the amorphous solute matrix are only partially understood. Two mechanisms, selective diffusion (Thijssen, 1971; King, 1971, 1970; Rulkens and Thijssen, 1972; Thijssen and Rulkens, 1968) and microregions (Flink and Karel, 1970, 1972), probably represent macro- and microviews of the same basic phenomena. The selective diffusion concept utilizes diffusional analysis on the system components to develop mathematical expressions by which volatile retention behavior during freeze-drying can be predicted; and structural aspects of the microregion concept allow qualitative explanation of volatile behavior during freeze-drying and reconstitution. The formation of microregions is caused by water crystallization, resulting in areas of highly concentrated food components and flavor volatiles. In the case of carbohydrates, flavor binding within these regions is largely through hydrogen bonding. Proteins and lipids, under such conditions of concentration, would bind flavor molecules similarly to the mechanisms described previously under B. Interaction of flavor volatiles with foods and food components.

C. Methods of assay

Earlier investigations relied on optical rotation, elemental analysis, conversion to picrate derivatives, melting points and frac-

tional distillations to separate and identify pyrazines (Maga and Sizer, 1973). In this review of pyrazine separation and identification techniques, the authors point out that, with recent developments in instrumentation, the pyrazine chemist can utilize gas liquid chromatography, mass spectrometry, ultraviolet spectrometry, infrared spectrometry, nuclear magnetic resonance and raman spectrometry to determine the composition of complex mixtures containing only trace amounts of each pyrazine. No mention, however, is made of the use or possible consideration of electrochemical techniques (for example, polarography) for the purposes of analysis of pyrazines in foods. Also, in their discussion of each instrumental method, very little emphasis is placed on the utilization of each technique for quantitative purposes, with the exception of gas liquid chromatography. This illustrates the lack of extensive research involving quantitative aspects of pyrazines in the aroma of potatoes and potato products (as well as other foods).

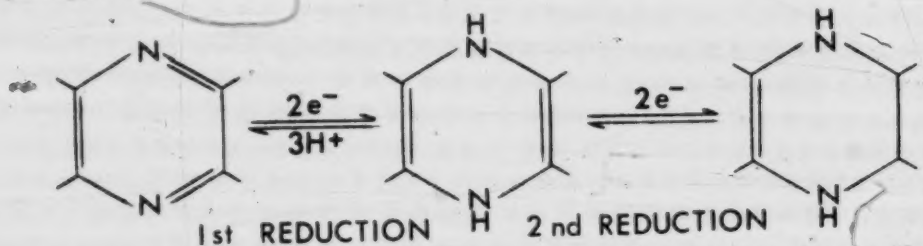
1. Gas liquid chromatography

Gas liquid chromatography (GLC) has been the predominant tool in the separation and, in some cases, the identification of pyrazines. In addition, pyrazines may be quantitated with this method, but this aspect has been given very little treatment with respect to the amounts of pyrazines found in the volatile constituents of raw and processed potatoes. To determine the levels of pyrazines in potato flavor, GLC has only been used on a semi-quantitative basis. Buttery *et al.* (1971), in characterizing the pyrazine and pyridine components of potato chip volatile oil, reported the approximate relative amount (l=relatively large; m=moderate; s=small) of each identified pyrazine, based on GLC analysis. Other studies reporting extremely low concentrations of

pyrazines in potatoes - Nursten and Sheen (1974), for example, reported a 2-methoxy-3-ethylpyrazine concentration in raw potatoes of less than 10 parts per 10^{12} - used odor threshold values and the concept of odor unit calculations as a basis for quantitative determination, rather than an instrumental detection technique. Considering the minute levels of pyrazines usually found in potato volatiles, it is absolutely necessary, for quantitative purposes, to have at hand a highly sensitive method of detection.

2. Direct current and differential pulse polarography

The pyrazine molecule is electrochemically active and capable of undergoing a reversible one- or two-stage reduction in the presence of hydrogen ions:



Thus, pyrazines may be detected polarographically, and can be easily quantitated in this manner since the amplitude of the signal (current, usually from the first reduction) received by the recorder is a direct function of the concentration of pyrazine in solution. In the polarographic technique, the entire reduction phenomenon is controlled solely by diffusion processes.

Although no literature exists pertaining to the application of polarography to pyrazine flavor chemistry in potatoes, pyrazines have been studied with the use of this method for several years. For example, Volke *et al.* (1965), in a classical paper on pyrazine electrochemistry, determined the polarographic reduction of pyrazine and its methyl de-

rivatives. Armand *et al.* (1974) also studied the electrochemical reduction of pyrazines into dihydropyrazines. Daniel *et al.* (1969), in one of the very few existing studies concerned with polarographic methods of assay in food flavors, quantitatively determined the levels of pyrazines in coffee.

The classical methods of polarographic determination rely on a voltage application in the direct current mode; by this method, quantitative results are obtained in the form of wave recordings, since the current arising from electroreduction is measured only once. For quantitative purposes, classical d.c. polarography compares favorably with gas liquid chromatography. The recent renaissance in electrochemical methods has produced a much more refined polarographic technique: differential pulse polarography. Here, voltage is applied in short pulses, and the diffusion current is measured twice, resulting in a peak rather than a wave and, thus, improved resolution. For quantitative measurements of pyrazines, differential pulse polarography is vastly superior to the classical GLC method, since a 10^4 times higher sensitivity may be achieved with differential pulse polarography (Marce and Hadziyev, 1977).

III. EXPERIMENTAL

Materials and Chemicals

Raw potatoes used were variety Netted Gem (Russet Burbank) grown in Southern Alberta. Specific gravity, as determined by the apparent loss of weight of tubers in water, was 1.098. Proximate analysis gave the following percentages: protein 2.5 (N x 7.5); fat, oil, 0.1; total carbohydrate, 19.4, of which 17.5 was starch; and crude fibre, 0.5. Vitamin contents after three months of storage at 4°C were in mg/100g dry matter: ascorbic acid, 60; thiamine, 0.4; riboflavin, 0.09; and niacin, 8.5. The content of ash, as determined by dry ashing at 450°C, was 1.6%. Alkali metals were the most abundant mineral constituents, with potassium and sodium present in levels of 1.3 and 0.18%, respectively. The chloride content on a dry weight basis, amounting to 0.044%, was very low regardless of locality. The average content in ppm of some transitional metals was determined by atomic absorption spectrophotometry to be: copper, 2.0; cobalt, 0.3; iron, 4.0; manganese, 2.5; nickel, 3.0; and zinc, 3.0.

Dehydrated potato granules produced from two different processing procedures were used. Those granules produced from a commercial scale add-back process (consisting of steam peeling, trimming, slicing, washing, pre-cooking, cooling, steam cooking, mashing by shearing action of the dried add-back granules against the hot potatoes, conditioning, remixing, air lift drying, fluidized bed drying, cooling and sifting) were prepared at Vauxhall Foods Ltd., Vauxhall, Alberta. The granules produced from a freeze-thaw technique were processed in our laboratory on a pilot plant scale. The freeze-thaw process consisted of the following steps: mechanical peeling, slicing, steam cooking, mashing with

a medium speed flat beater, freezing in an air blast freezer at -20°C, thawing at room temperature, pre-drying, granulation, drying, cooling and sifting (Ooraikul, 1973). Some further details regarding this process are presented and discussed under Methods. Some characteristics of the end-product granules obtained from both processes were as follows: moisture content, approximately 7%; broken cell count, 2% for freeze-thaw, and 5% for add-back granules; total starch content, 83.7%, with an average amylose content of 21.2%; and free starch, expressed as Blue Value Index, 123 for freeze-thaw, and 90 for add-back granules. Both types of granules contained the following: sodium bisulfite, 350-550 ppm (as SO_2); reducing sugar, 2%; maximums of sodium acid pyrophosphate, 0.3%; emulsifier, 0.5%; and phenolic antioxidant, BHT, approximately 10 ppm. The bulk densities were 0.86-0.94 and 0.77-0.83 g/ml for add-back and freeze-thaw granules, respectively.

Two commercial brands of dehydrated instant mashed potato flakes (Carnation and Shirriff) were used, and these were supplied by local retail supermarkets. Brand A flakes (Carnation) contained the following ingredients: potatoes, glycerol monostearate and, as added preservatives, sodium acid pyrophosphate, sodium bisulfite and butylated hydroxytoluene (BHT). Brand B flakes (Shirriff) had as their ingredients: potatoes, monoglycerides, sodium bisulfite, sodium sulfite, tetra sodium pyrophosphate, citric acid, BHA, BHT and imitation flavor.

Pure potato matrix components were obtained from various sources. Pectin, molecular weight 180,000-300,000, and pure potato starch, pregelatinized and suitable for chromatographic use, and containing approximately 75% amylopectin and 25% amylose, were obtained from Sigma Chemical Co, St. Louis, Mo. Potato amylose, with a molecular

weight of over 150,000, was purchased from Aldrich Chemical Co. Inc., Milwaukee, Wis. Amylopectin, with a molecular weight of approximately 1,000,000, and cellulose, microcrystalline, with a particle size of less than 100μ, and suitable for use in thin layer chromatography, were obtained from J.T. Baker Chemical Co., Chicago, Ill. Pure potato protein was not commercially available. Thus, a method was developed for extraction of total protein from raw potato tubers; this procedure is discussed later under Methods. The resulting protein was ≥95% in purity, with a non-protein nitrogen (NPN) content of about 50%. The true protein fractions had molecular weights ranging from 10,000 to 150,000 (Golan and Hadziyev, 1977).

A chemically unspecified commercial sample of imitation potato flavor mixed with a powdered carrier was obtained from Polack's Frutal Works Inc., Middletown, N.Y.

Methylpyrazine (b.p. 134-5°C); 2,5-dimethylpyrazine, 2,6-dimethylpyrazine, and 2,5- and 2,6-dimethylpyrazine (b.p. 59-60°C); 2,3,5-trimethylpyrazine (b.p. 80-1°C); 2-methyl-3-ethylpyrazine (b.p. 77-8°C); 2-methyl-5-ethylpyrazine and 2-methyl-6-ethylpyrazine (b.p. 80-1°C); and 2-ethyl-3,5-dimethylpyrazine and 2-ethyl-3,6-dimethylpyrazine - all of ≥99% purity - were obtained from Pyrazine Specialties, Atlanta, Ga. High purity 2-methoxy-3-ethylpyrazine (≥98%, b.p. 52-4°C, n_D^{20}, 1.5019 ±0.0005) was purchased from Eastman Organic Chemicals, Rochester, N.Y.

Chromosorb W (acid washed), 60/80 mesh, was obtained from Applied Science Laboratories Inc., State College, Pa.; and Carbowax 1500 (maximum temperature 200°C) was obtained from Chromatographic Specialties Ltd., Brockville, Ont. Quaternary methyl- or butyl ammonium bromides or hydroxides were purchased from Aldrich Chemical Co. Inc.,

Milwaukee, Wis. Gelatin was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio. All other chemicals, of reagent grade or higher, were supplied by Fisher Scientific Co., Fair Lawn, N.J.

Equipment

Earlier polarographic work (direct current mode only) was performed on a Sargent Model XV Polarograph (Chicago, Ill.). A Princeton Applied Research Model 174A polarographic analyzer (Princeton, N.J.), in a direct current and differential pulse mode, was used in conjunction with a Houston X-Y recorder for the majority of polarographic determinations. Gas liquid chromatographic analysis was carried out with a Bendix Model 2500 Chromatograph (Bendix Corp. Process Instr. Div., Ronceverte, W.Va.). Dehydration of potato mash was done in a stirred fluidized bed drier (modified Manesty Petrie 10E, from Manesty Machines Ltd., Liverpool, England). Potatoes were mashed in a Kitchen Aid Model K5-A Medium Speed Masher-Beater (Hobart Mfg. Co., Troy, Ohio) and dehydrated granules were sifted in a Tyler Portable Sieve Shaker (W.S. Tyler Co. Can. Ltd., St. Catharines, Ont.). Potato cake was pressed on a Carver Laboratory Press (Fred S. Carver Inc., Summit, N.J.). Centrifuges used were: 1) Sorvall SS-1 Superspeed Angle Centrifuge, manufactured by Ivan Sorvall Co. Inc., Norwalk, Conn., and 2) Beckman Model J21B Refrigerated Centrifuge, Beckman Instr. Inc., Palo Alto, Calif. The peristaltic pump employed in column chromatography was a Buchler Instruments Polystatic Pump (Buchler Instruments, Fort Lee, N.J.) equipped with a silicone rubber tubing of 1.1 mm i.d. (LKB Produkter, AB, Bromma, Sweden). The pump was used in conjunction with an ISCO Model 328 Golden Retriever Linear Fraction Collector from Instrumentation Specialties Co., Lincoln, Neb. Pyrazine recovery by solvent

evaporation was accomplished with a Büchi Model VE 50 Rotary Vacuum Evaporator obtained from Rinco Instr. Co. Inc., Greenville, Ill. Moisture determinations were carried out in a National Appliance Co. Vacuum Oven, and pH measurements were made on a Fisher Accumet Model 230 pH/Ion Meter (Fisher Scientific Co. Ltd., Fair Lawn, N.J.).

Methods

A. Processing effect on pyrazine retention

1. Potatoes and potato products

a. Freeze-thaw process

Pyrazine incorporation

Raw potatoes were peeled, washed, sliced and steam cooked for 35 min. Following these steps, a 75g sample of cooked potato was immediately removed and frozen at -20°C for pyrazine analysis. Monoglyceride was added to the cooked potatoes to give 0.5% emulsifier, and the mix was mashed at high speed for 1.5 min in a medium speed flat beater. Subsequent to mashing, another 75g sample was immediately removed and frozen, and the remaining mash was cooled to room temperature.

In order to follow the retention of methyl-, 2,3,5-trimethyl-, 2-methyl-3-ethyl- and 2-methoxy-3-ethylpyrazine during the freeze-thaw process, the synthetic pure pyrazines were incorporated into the cooled mash at a level of 1g/kg mash. The pyrazines, as 1% aqueous solutions, were thoroughly mixed into the mash with a wooden spoon, after which the mash was spread 0.5 in. thick on stainless steel trays, which were then covered with aluminum foil and frozen in an air blast freezer (air velocity 3,000 cu ft/min, minimum air temperature of -20°C) for several hours, and then thawed at room temperature. After the mash was uniform-

ly thawed, the dehydration process was carried out in the bowl of a stirred fluidized bed drier. The bowl was tightly covered with a nylon collecting bag, and the moist mash was pre-dried with the stirrer speed set at 20 rpm, air flow at 373.2 ft/min, and the inlet air temperature adjusted to 49°C. Following the pre-drying step was granulation, in which the air velocity was reduced to 175.6 ft/min, the air temperature reduced to 37.5°C, and the stirrer speed increased to 480-500 rpm. Finally, the drying stage was performed in hot air with a temperature of 54.5°C and a velocity of 231.7 ft/min. When the temperature of the granules reached that of the drying air, the heaters were turned off and the granules were allowed to cool. Cooled granules were sifted and those particles larger than 60 mesh in size were discarded. At the end of each step in the dehydration procedure, samples were removed for pyrazine analysis (10g) and moisture determination (5g). For analysis of initial pyrazine and moisture contents of the cooled mash (prior to dehydration), 25g samples were removed for the former and 5g samples taken for the latter.

Pyrazine recovery

Pyrazine extraction from potato samples having the initial natural concentration (steam cooked and steam cooked, mashed potato), the initial synthetic pyrazine concentration (enriched mash), and those enriched with synthetic pyrazines and subsequently pre-dried, granulated and dried, differed only in the sample size: 75, 25 and 10g, respectively.

The samples were extracted in an Erlenmeyer flask with dichloromethane (5:1 v/w) by continuous shaking for 10-15 min, followed by filtration through Whatman No. 2 paper. The remaining residue on the

filter paper was re-extracted with an equal volume of solvent. Both extracts were combined and transferred to a round bottom flask containing exactly 25 ml of 2N acetate buffer (pH 4.90 ± 0.01). The organic solvent was then evaporated under aspirator vacuum at room temperature in a Büchi rotary evaporator. The remaining aqueous phase containing the pyrazines was clarified by centrifugation at 18,400xg in polyethylene tubes, using a Sorvall centrifuge. The supernatant was separated from the yellow sediment, filtered again through Whatman No. 2 paper, and then transferred to a polarographic cell for pyrazine content determination.

Pyrazine detection and quantitation

(i) Direct current polarography

Initial studies on solvent extraction of pyrazines were carried out with the Sargent XV polarograph, which can be used for direct current polarography only. The form of the potential applied in a d.c. run is shown in Figure 1. The cell assembly used in conjunction with the Sargent polarograph consisted of a saturated calomel electrode (SCE) connected by a salt bridge to the test solution chamber containing the dropping mercury electrode (DME), and is shown in Figure 2. The following operating conditions were used: scan direction, negative, with a range of 1.5V and initial potential of 0.0V; drop time, 2-5 sec; current sensitivity for a full scale recording, 0.003 to 0.100μa/mm; and damping, 2. One drop of a 1% gelatin solution was added to give a final gelatin concentration of 0.005% for maxima suppression.

Subsequent to solvent extraction experiments, the Princeton 174A polarographic analyzer was employed for those determinations requiring d.c. polarography. The cell assembly for this 2-electrode mode

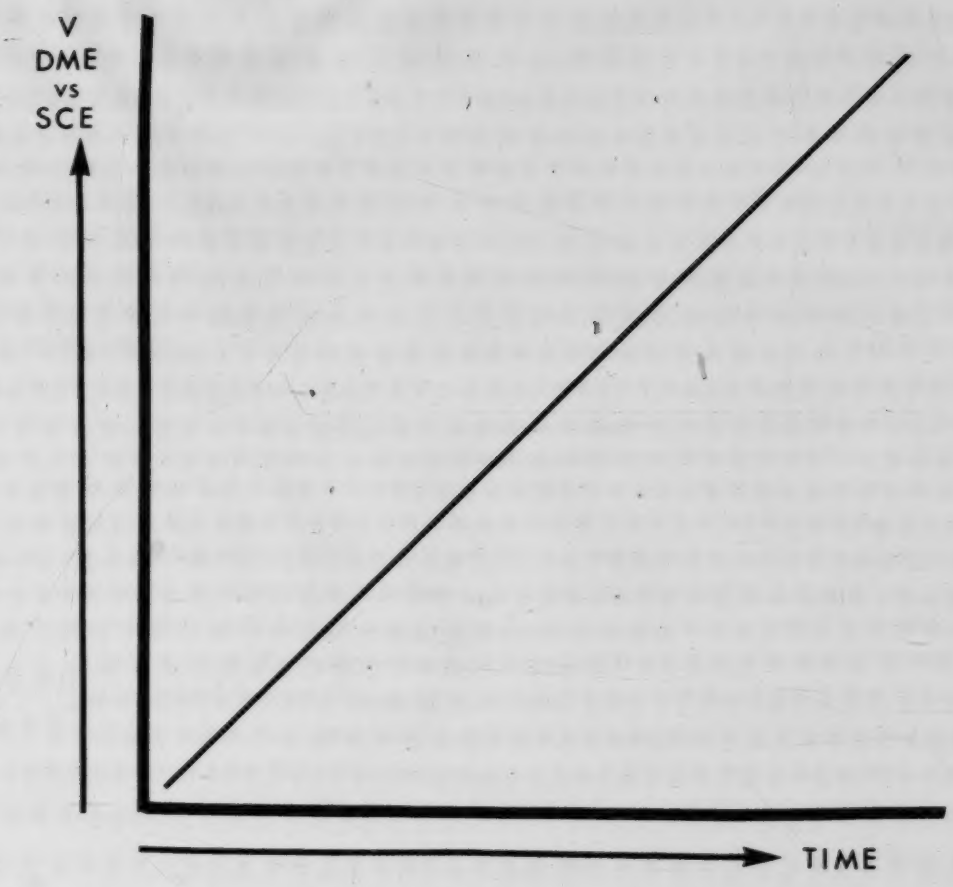


Figure 1. Potential application in the direct current mode. Voltage was applied along a slow linear ramp.

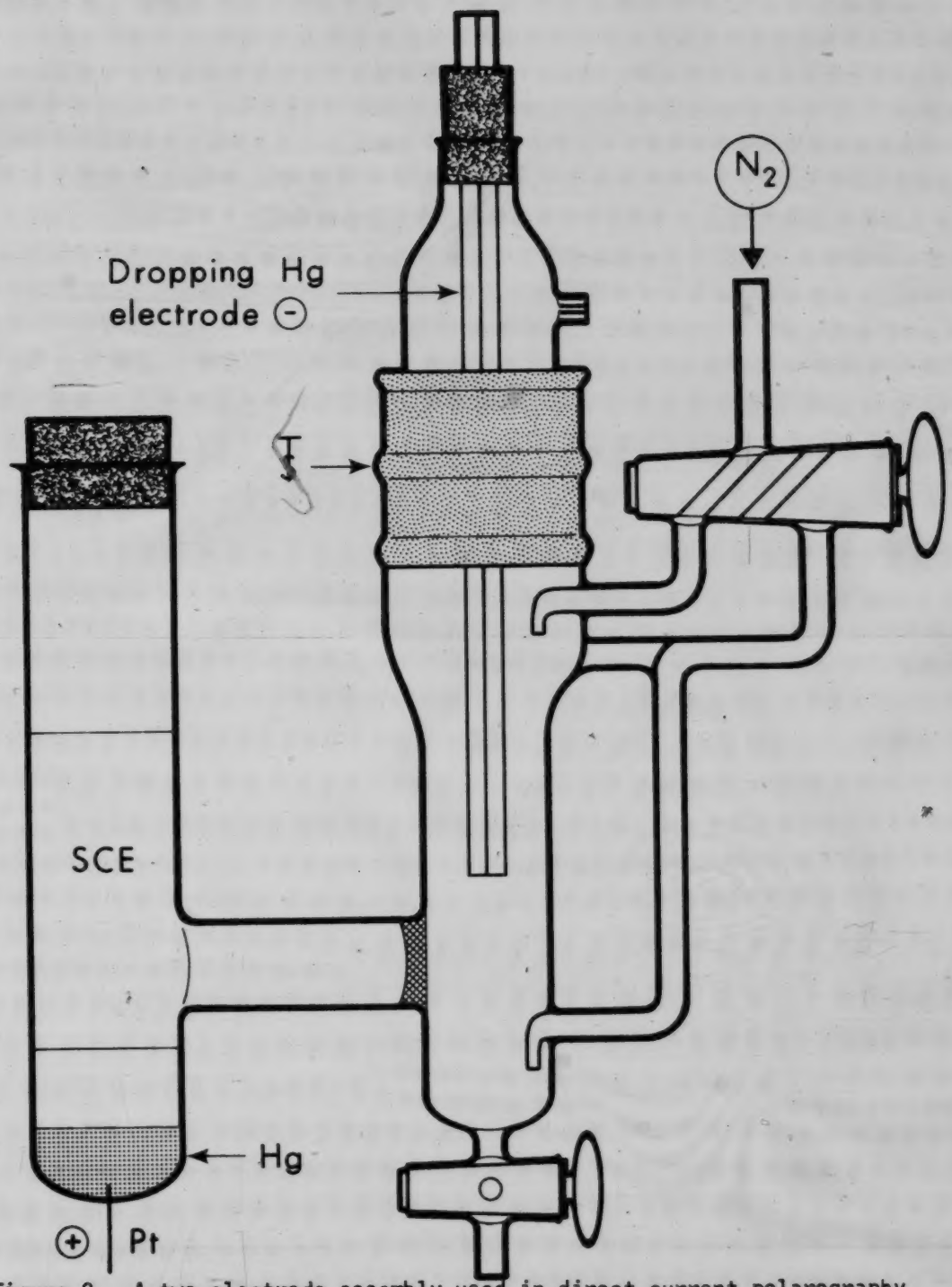


Figure 2. A two-electrode assembly used in direct current polarography. DME, dropping mercury electrode with a capillary tube i.d. of 0.05-0.08 mm (E.H. Sargent), connected by salt bridge to the SCE (saturated calomel electrode) fitted with a Pt (platinum) wire.

consisted of a micro SCE electrode and a DME electrode attached to a drop knocker (Figure 3). The platinum counter electrode is not used in the d.c. mode. Voltage application was identical to that in Figure 1, and operating conditions were as follows: potential scan rate, 2mV/sec; scan direction, negative, with a range of 1.5V and initial potential of 0.0V; drop time, 2 sec; sensitivity for a full scale recording, 0.2ma (8×10^{-4} mA/mm); and low pass filter time constant, 3 sec. Maxima were suppressed with gelatin (0.005% concentration in solution).

(ii) Differential pulse polarography

This mode was used predominantly and was utilized at low pyrazine concentrations, when the capacitive current of d.c. polarography limited the sensitivity. The method of potential application is shown in Figure 4. A small voltage pulse of 50mV was applied for 56.7 msec on the normal voltage ramp, near the end of the drop time. The measured current value for a duration of 16.7 msec was the difference in currents flowing just before the pulse was applied and before the pulse was removed. This timing kept the capacitive current influence at a minimum, while the faradic current, due to pyrazine reduction, was at its maximum. In addition, the differential mode provided peaks rather than waves, which increased the resolution and accuracy.

The polarograph was the Princeton 174A analyzer, but in the differential pulse mode. The electrode set-up had, as did the d.c. mode of the 174A analyzer, a drop knocker assembly. The cell also contained a Pt wire counter electrode (Figure 3), the third electrode being required for potentiostatic control (Figure 5). Operating conditions were: potential scan rate, 2mV/sec; scan direction, negative, with a range of 1.5V and initial potential of 0.0V; drop time, 1 sec; modulation amp-

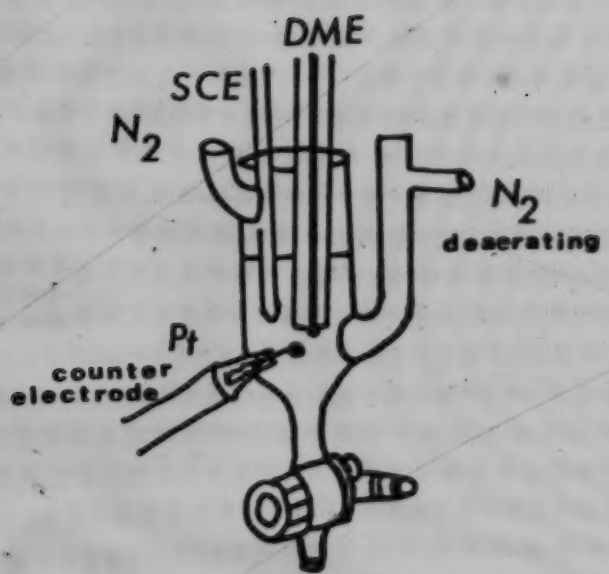


Figure 3. A three-electrode assembly used in differential pulse polarography. DME, dropping mercury electrode with a capillary tube i.d. of 0.05-0.08 mm (E.H. Sargent); SCE, saturated calomel electrode, fiber junction type; and Pt, platinum wire, 1 mm, placed 0.5 cm from the DME, and used as an auxiliary electrode.

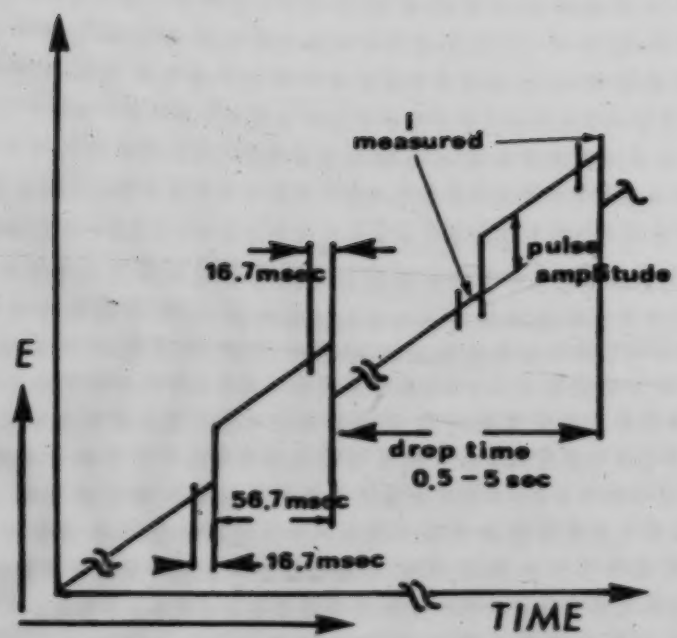


Figure 4. Differential pulse excitation waveform. It consisted of a slow linear potential ramp upon which the 50mV voltage pulses (duration of 56.7 msec) were superimposed.

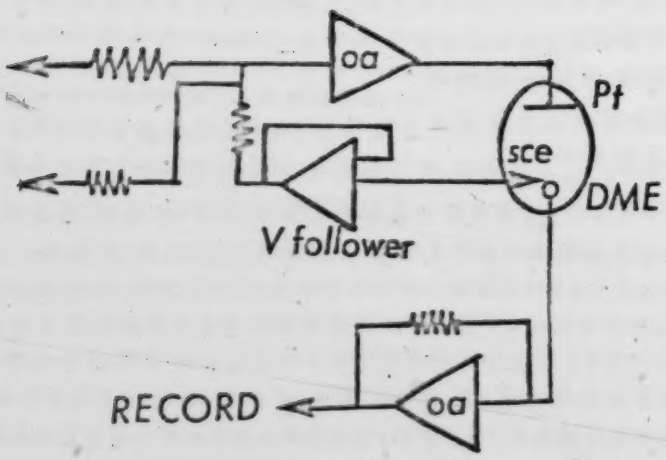


Figure 5. Potentiostatic control in a three-electrode circuit. In differential pulse polarography, voltage is controlled by the cathodic follower.

litude, 50mV; low pass filter, off; and current range for a full scale recording (250 mm), 0.1μA to 0.1 mA. Gelatin, 0.005% in solution, was again required for maxima suppression.

(iii) Gas liquid chromatography

Gas chromatography runs were performed on a Bendix 2500 chromatograph. An FID detector was used and the chromatograph was equipped with "U" shaped glass columns of 170 x 0.4 cm i.d. packed with 10% Carbowax 1500 on Chromosorb W (AW) 60/80 mesh. The carrier gas was nitrogen, with a flow rate of 30 ml/min, and runs were conducted isothermally at 105°C.

2. Potato matrix components

a. Freeze-thaw process

The following pure matrix components of the potato cell were enriched with pure synthetic pyrazines and then subjected to the freeze-thaw process described previously: starch, amylose, amylopectin, pectin and cellulose. Because of insufficient quantities available for processing on a pilot plant scale, potato protein was not examined for pyrazine retention during freeze-thaw dehydration. The matrices were enriched with the same four pyrazines used before for flavor enrichment of potato mash. Pyrazines were again incorporated, as in potato mash, at a level of 1g/kg matrix. These compounds, as 1% solutions, were mixed thoroughly into each matrix with a wooden spoon, after which the mix was frozen and thawed as mentioned earlier.

In order to promote ease of fluidization of each component, low initial moisture levels were required. Thus, times of pre-drying, granulation and final drying were substantially decreased. However, the drying parameters of stirrer speed, air temperature and air velocity

were identical to those used during dehydration of moist potato mash. Following each step in the drying process, 10g samples were recovered for pyrazine analysis and 5g samples taken for moisture determination. Samples of 25g were used to determine the initial synthetic pyrazine concentration of enriched matrices prior to processing, and initial moisture contents were evaluated with 5g samples.

The recovery of pyrazines from the matrices was carried out with the same technique employed previously, with the exception being that the centrifugation and filtration steps following rotary evaporation were not required for the isolation of pyrazines from these components.

Pyrazine quantitation was achieved by the method outlined under Differential pulse polarography.

B. Model systems of potato components for study of pyrazine retention

A column chromatographic technique, consisting of solvent extraction of pyrazines from packed columns of pure potato starch, amylose, amylopectin, pectin, cellulose and protein, as well as add-back dehydrated potato granules, was developed in order to study the flavor interactions, in model systems, with purified cellular constituents and whole potato cells (granules).

All of the components were commercially available except potato protein. Thus, a method was devised for protein extraction from raw tubers, and this procedure was a modification of those used by Osborne and Campbell (1896), Xander and Hoover (1959), and Hoff (1975). In addition, the extracted proteins were broadly characterized based on extraction methods suggested by Osborne and Campbell (1896), and Desborough and Weiser (1972).

Fresh potatoes were peeled, washed, grated and blended at low

speed for 10-30 sec in a Waring blendor containing 10-20 ml of 0.5% sodium sulfite and 2 ml of 0.0004% EDTA. The grated potatoes were oven dried, powdered and then analyzed by the standard micro Kjeldahl method (A.O.A.C., 1975) for total nitrogen content of fresh potato tissue.

The potato blend was strained through cotton cloth into a beaker containing a few ml each of the sodium sulfite and EDTA. The potato juice was centrifuged at 11,000xg in polyethylene bottles for 20 min, after which the supernatant was removed, and NaCl added to make a 10% salt solution. The resulting solution was heated at 80-90°C for 30 min, cooled and again centrifuged at 11,000xg for 20 min. The supernatant was discarded and the residue was washed with 90% ethanol, filtered by aspirator vacuum, washed with acetone and air dried.

The moist cake remaining in the cotton cloth after juice expulsion was pressed in a hydraulic press at 25,000 psi, and the dry cake was then subjected to micro Kjeldahl analysis. Also, samples (10g) of the dry cake were extracted, by mechanical shaking, with 30 ml each of the following solvents: tap water, 10% NaCl, 70% ethanol and phenol: acetic acid:water (2:1:1, v/v/v). Each extract was centrifuged (polyethylene tubes for water, NaCl, ethanol extracts and stainless steel tubes for phenol extract) at 10,000xg for 10 min, after which the pellets were resuspended and extracted three more times. The remaining pellets were analyzed for cake nitrogen by the micro Kjeldahl method.

1. Pyrazine application

Glass chromatographic columns, 29.5 x 1.2 cm i.d., were uniformly packed with the matrix components (5-15g) to give a constant column volume of 15 ml. Neat methyl-, 2,3,5-trimethyl-, 2-methyl-3-ethyl- and 2-methoxy-3-ethylpyrazine were diluted in tubes stoppered

with rubber septums with anhydrous diethyl ether to give two solutions of concentrations that, upon application to the column and ether evaporation, would be 1 and 10 ppm pyrazine with respect to the solid matrices. Before pyrazine application, the columns were equilibrated for at least 2 hr with water-saturated dichloromethane (0.7% H_2O, pH 5.35), fed into the column by a Buchler peristaltic pump fitted with narrow silicone tubing. The pyrazine-ether solutions (100 µl) were then injected with a micro syringe to a depth of approximately 0.5 cm below the surface of the column packing.

2. Pyrazine recovery

After the diethylether was allowed to evaporate (approximately 1 min), the peristaltic pump was turned on, and the pyrazines were eluted with water-saturated dichloromethane flowing at a constant rate of 0.90 ml/min. Twenty fractions of 2 ml each were collected by the fraction collector, after which the column was washed for 2 hr with the solvent system.

3. Pyrazine quantitation

All fractions collected were well shaken for 1 min, and then analyzed for pyrazine content with the aid of the polarographic method elucidated earlier under Differential pulse polarography.

IV. RESULTS

A. Pyrazine retention during processing

1. Potatoes and potato products

a. Freeze-thaw process

Methodology of pyrazine determination

The extent of pyrazine recoveries from aqueous solutions, using selected organic solvents, is presented in Table 1. A transfer of py-

Table 1. Recovery in percent of methylpyrazine from its aqueous solution using organic solvents as extractants.

Solvent	Extraction (1:1 v/v)		
	1st	2nd	3rd
Chloroform	92.6*	99.2	99.9
Dichloromethane	88.4	94.9	99.9
Diethyl ether	59.1	80.9	91.6
Ethyl acetate	72.2	84.7	93.1
Hexane	51.0	74.2	90.0

*Results determined from difference of cathodic wave heights at $E_{1/2}$ -0.75V before and after extraction.

razines into organic solvent to an extent of at least 94% was achieved readily by two extractions with dichloromethane. Chloroform proved even better than dichloromethane in extracting pyrazines from aqueous phase, with a 99.2% recovery obtained after only two extractions. Diethyl ether, ethyl acetate and hexane displayed a much less powerful ability to remove pyrazines from aqueous solutions than the two chlorinated solvents. Hexane was the weakest of the five solvents studied in this respect, and diethyl ether, the most volatile solvent of those tested (with a vapor pressure much higher than that of most pyrazines), also proved to be inadequate for quantitative pyrazine recovery. As an extracting solvent in subsequent work with potatoes and potato products and with model systems, dichloromethane was selected over chloroform, due to the low boiling point of the former solvent.

The possibility of re-extraction of pyrazines from organic solvents into an aqueous phase, by using pH 1 water (1N HCl) as an extractant, was suggested by Reineccius *et al.* (1972). The authors claimed that, following re-extraction of methylpyrazine, dimethylpyrazine and tetramethylpyrazine with pH 1 water from diethyl ether originally containing 100 µg each of the pyrazines, only 5 µg or less of each pyrazine remained in the pooled diethyl ether phases. This represents a re-extraction efficiency of at least 95%.

The procedure of aqueous recovery of pyrazines from organic phases with a low pH aqueous solvent was investigated in this study (Table 2) as an attempt to avoid the required evaporation of organic solvent, since the possibility of pyrazine loss through vaporization would exist. The results, however, based on methylpyrazine, showed that, even after a third extraction, the pH 1 water of Reineccius and

Table 2. Aqueous recovery* in percent of methylpyrazine from organic solvents.

Solvent	Re-extraction (1:1 v/v)		
	1st	2nd	3rd
Chloroform	2.3**	2.5	4.1
Dichloromethane	1.0	5.1	7.8
Diethyl ether	2.2	3.4	4.2
Ethyl acetate	1.9	2.0	6.3
Hexane	5.3	5.4	8.9

*Based on re-extraction with pH 1 water (1N HCl).

**Results determined from difference of cathodic wave heights at $E_{1/2}$ -0.75V before and after extraction.

workers did not remove more than 10% of this pyrazine from any of the five organic solvents tested.

Thus, since the recovery of pyrazines from organic to aqueous phase was inadequate, the possibility of removing the organic phase by evaporation was also examined. In order to minimize pyrazine losses, the solvent evaporation had to be carried out at room temperature or lower. Dichloromethane, because of its low boiling point, can be vaporized at room temperature under aspirator vacuum and, when substantial quantities are evaporated, the temperature becomes significantly lower than that of room temperature, due to the phenomenon of evaporative cooling.

Pyrazine losses, as determined from recovery tests involving potato samples (by extraction twice with dichloromethane in a ratio of 5:1 v/w and evaporation of the extract in the presence of acetate buffer), are presented in Table 3. As seen from these results, the major losses of pyrazines were experienced in the evaporation step and they followed the boiling points of the pyrazines. Losses observed during the organic solvent extraction step were low enough to be considered insignificant. The evaporative loss of pyrazines was considerable; however, in spite of this, the loss for the lowest boiling, 2-methoxy-3-ethylpyrazine, was not higher than 18%, which was considered as acceptable. Calculation of the correction factor for the evaporation loss was used for the final results.

Despite the fact that pyrazine losses during vaporization of dichloromethane were considered low enough to be acceptable, the possibility of polarographic detection and quantitation of pyrazines in organic phase was studied. Haydar and Hadziyev (1974) successfully

Table 3. Pyrazine losses in percent in the extraction procedure applied.

Pyrazine incorporated into potato	Pyrazine b.p. °C	Potato sample extraction step	Organic solvent evaporation step	Total loss
Methylpyrazine	134-5	0.11	7.3	7.4
2,3,5-Trimethyl-pyrazine	80-1	0.02	7.2	7.2
2-Methyl-3-ethyl-pyrazine	77-8	0.03	10.7	10.7
2-Methoxy-3-ethyl-pyrazine	52-4	0.04	17.9	17.9

quantitated lipid peroxides in an organic solvent system of methanol: benzene (1:1 v/v), with 0.3M LiCl as the supporting electrolyte. However, when this system was applied to the detection of pyrazines, it was found that pyrazine resolution was unattainable, since their reduction waves were in close proximity to or overlapping with the electrolyte diffusion current. Thus, separation of pyrazine and electrolyte reductions was not possible even at relatively high pyrazine concentrations. Besides LiCl, several other electrolytes were tested, including 0.3M KCl, and quaternary methyl- or butyl ammonium bromides or hydroxides; each electrolyte gave rise to a reduction potential very near to the major reduction wave of methylpyrazine. In addition to this phenomenon, large shifts in half-wave potentials ($E_{1/2}$'s) towards more positive potentials (-0.2 to -0.6V), with accompanying peak broadening, were observed when pyrazine currents were recorded in organic solvents instead of aqueous buffer solution.

The $E_{1/2}$'s of pyrazine peaks and waves recorded in 2N acetate buffer, pH 4.90, are listed in Table 4. All substituted pyrazines assayed had a major reduction occurring between $E_{1/2}$ -0.78 to -0.94V, which corresponded to a two electron (2e) cathodic process resulting in reduction of substituted pyrazines into dihydropyrazines (Volke *et al.*, 1965; Daniel *et al.*, 1969). The major wave was then followed by more negative waves, the number of additional reductions appearing to be a function of the nature of ring substitution. Substitution by methyl or ethyl groups made the reduction more difficult, as reflected by a 39 to 44 mV shift towards a more negative $E_{1/2}$ for the major peaks. The highest $E_{1/2}$ was experienced with 2-methoxy-3-ethylpyrazine (Volke and Beran, 1975). The properties of the more negative waves, in which the

Table 4. Half-wave potentials ($E_{1/2}$) for some substituted pyrazines in 2N acetate buffer, pH 4.90, obtained by direct current (d.c.) and differential pulse (d.p.) polarography.

Substituted pyrazine		Cathodic (-) waves 1st	2nd	3rd	4th
Methylpyrazine	d.c.	0.794*	1.275**	-	-
	d.p.	0.781	1.265**	-	-
	mV	13	10	-	-
2-Methyl-3-ethylpyrazine	d.c.	0.828	1.225**	-	-
	d.p.	0.820	1.212**	-	-
	mV	8	13	-	-
2,3,5-Trimethylpyrazine	d.c.	0.837	1.181	1.260	1.456
	d.p.	0.825	1.175	1.250	1.445
	mV	12	6	10	11
2-Methoxy-3-ethylpyrazine	d.c.	0.935	1.106	1.260	1.455
	d.p.	0.921	1.090	1.254	1.441
	mV	14	16	6	14

*All $E_{1/2}$ values are vs SCE.

**Non-diffusion controlled; in all runs, the acetate buffer used as an inert supporting electrolyte had its diffusion current commencing at -1.5V. The polarographic maxima, with the exception of trimethylpyrazine, were readily suppressed by added gelatin.

pyrazines undergo a further reduction, most probably to the tetrahydroderivatives (Armand *et al.*, 1974), appeared to be different. Some of the waves lost, while others retained, their diffusion controlled character. Therefore, wave height, as a linear function of pyrazine concentration, was retained by trimethyl- and 2-methoxy-3-ethylpyrazine, but was lost with methyl- and 2-methyl-3-ethylpyrazine and, thus, in quantitative analysis, the height of the first wave was used (Figure 6). Also, as seen in Table 4, the $E_{1/2}$'s obtained in the differential pulse mode did not correspond to those recorded by d.c. polarography. The latter values were 6-16 mV more negative, which was not unexpected since the d.c. mode did not compensate for the voltage drops imposed by resistance of the test solution and SCE (see Figure 7).

Sensitivity of pyrazine detection also varied. In the d.c. mode, using the highest amplification (3×10^{-3} μA/mm), which still provided a wave relatively free of background noise, the detection limit was 1×10^{-7}M. Highly accurate recordings were in a concentration range of 0.2 to 10×10^{-6}M. Using the same amplification, the differential pulse mode was about 10^2 times more sensitive, with the greatest accuracy in the range of 0.1 to 9×10^{-6}M (see Figure 8).

Pyrazines are easily separated and identified utilizing the classical method of gas liquid chromatography. This is illustrated in Table 5, which presents retention data for pyrazines with various ring substitutions. As can be seen, the retention time increases with an increasing degree of substitution (larger molecules). Although the GLC method has been the most popular technique used in pyrazine detection, the method of differential pulse polarography proved to be far superior for purposes of pyrazine quantitation, since gas liquid chromatography

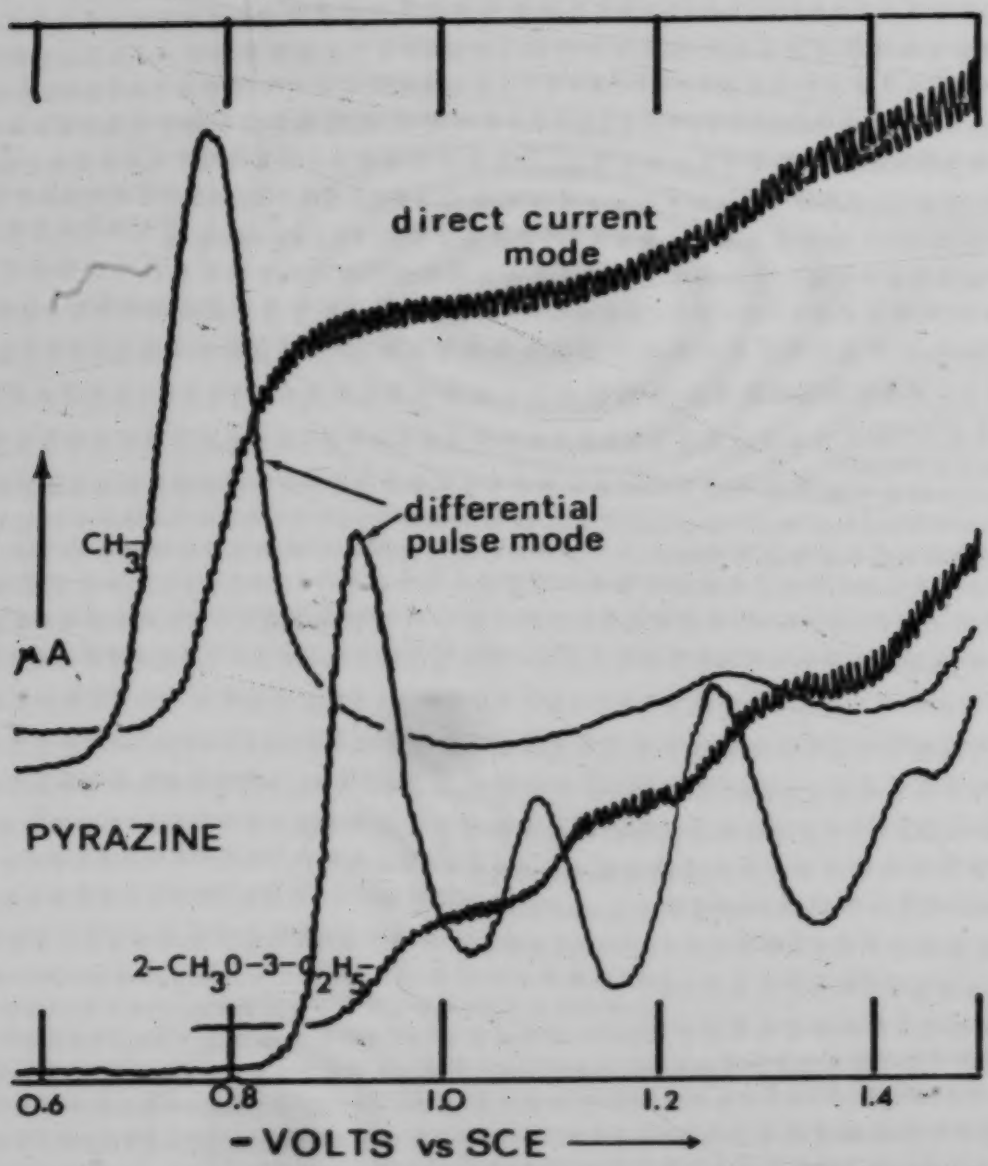


Figure 6. Polarographic recordings in direct current (d.c.) and differential pulse (d.p.) mode of methylpyrazine and 2-methoxy-3-ethylpyrazine. The height of the first peak obtained in d.p. mode was used for quantitative calculations.

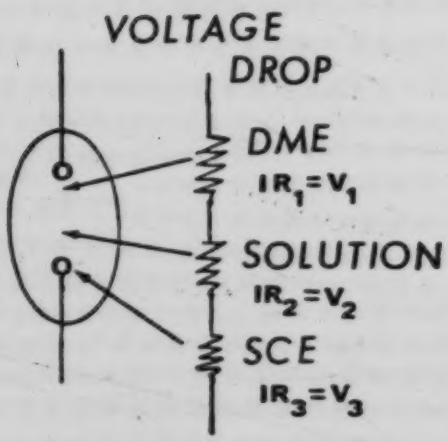


Figure 7. A d.c. polarography electrode cell, which does not compensate for voltage drops within the solution and at the SCE.

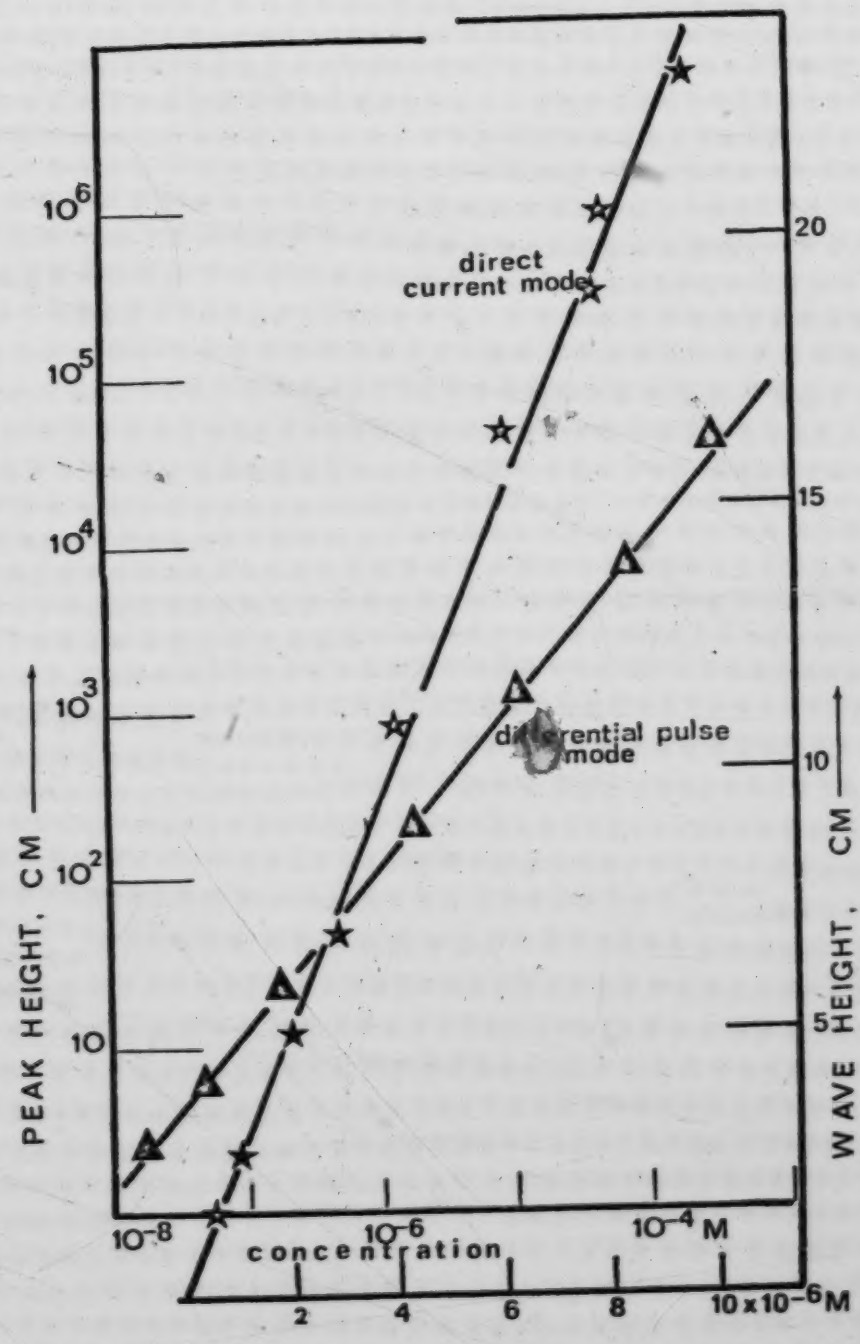


Figure 8. Peak and wave heights vs concentration of methylpyrazine. Obtained by d.p. and d.c. polarography in 2N acetate buffer, pH 4.90, at a sensitivity of 0.002 μA/mm. The linear responses within the range of 1 x 10^{-6}M to 0.1 x 10^{-8}M in d.p. mode and 1 - 10 x 10^{-6}M in d.c. mode were used as calibration curves.

Table 5. Retention time values in min for some substituted pyrazines separated in a column packed with 10% Carbowax 1500 on Chromosorb W 60/80 mesh.

Substituted pyrazine	Retention time*	
	1st peak	2nd peak
Methyl-	3.83**	-
2,5-Dimethyl-	4.90	-
2,5- and -2,6-Dimethyl-	5.22	-
2-Methyl-3-ethyl-	7.34	-
2,3,5-Trimethyl-	7.45	-
2-Ethyl-3,5- and -3,6-dimethyl-	9.20	9.95

*GLC runs conducted isothermally at 105°C. Carrier gas (N_2) had a flow rate of 30 ml/min.

**In all runs, pyrazine concentration was 1×10^{-5}M.

had a detection limit no better than 1 x 10^{-7}M.

Pyrazine retention during granule production

During dehydration of moist mash in the freeze-thaw process, the highest temperatures of the drying air, recorded at the base of the fluidized bed drier and at the exhaust pipe, were 86° and 60°C, respectively (Figure 9). The top curve in Figure 9 represents the temperature profile of the inlet air, while the temperature of the granules recorded continuously is shown in the lower profile. Despite the higher temperatures of the drying air, the granules had an average temperature of only 35°C, which rose for a short time to 76°C, but never exceeded 85°C. The heating portion of the dehydration process was stopped at the point where granule temperature reached drying air temperature, in this case 76°C.

Synthetic pyrazines, which were incorporated into the moist mash, were readily vaporized throughout the entire dehydration process. Losses of each of the four pyrazines studied, during the pre-drying, granulation and final drying steps of the procedure, are represented in Table 6. The initial moisture content of the mash, after incorporation of 1% aqueous pyrazine solutions, was 79.36%. As seen from Table 6, the major loss of pyrazines, which amounted to an average of 65.7% for methylpyrazine, and up to 88% for 2-methyl-3-ethylpyrazine, occurred during the pre-drying step. In granulation, completed at a moisture content of 20.1 to 36.0%, additional losses of 14.3 to 18.3% were found, with the exception of methylpyrazine (29.8%). Final drying brought about an end-product in which the cumulative loss of all added pyrazines was 99.9%.

The extent of pyrazines removed throughout the dehydration pro-

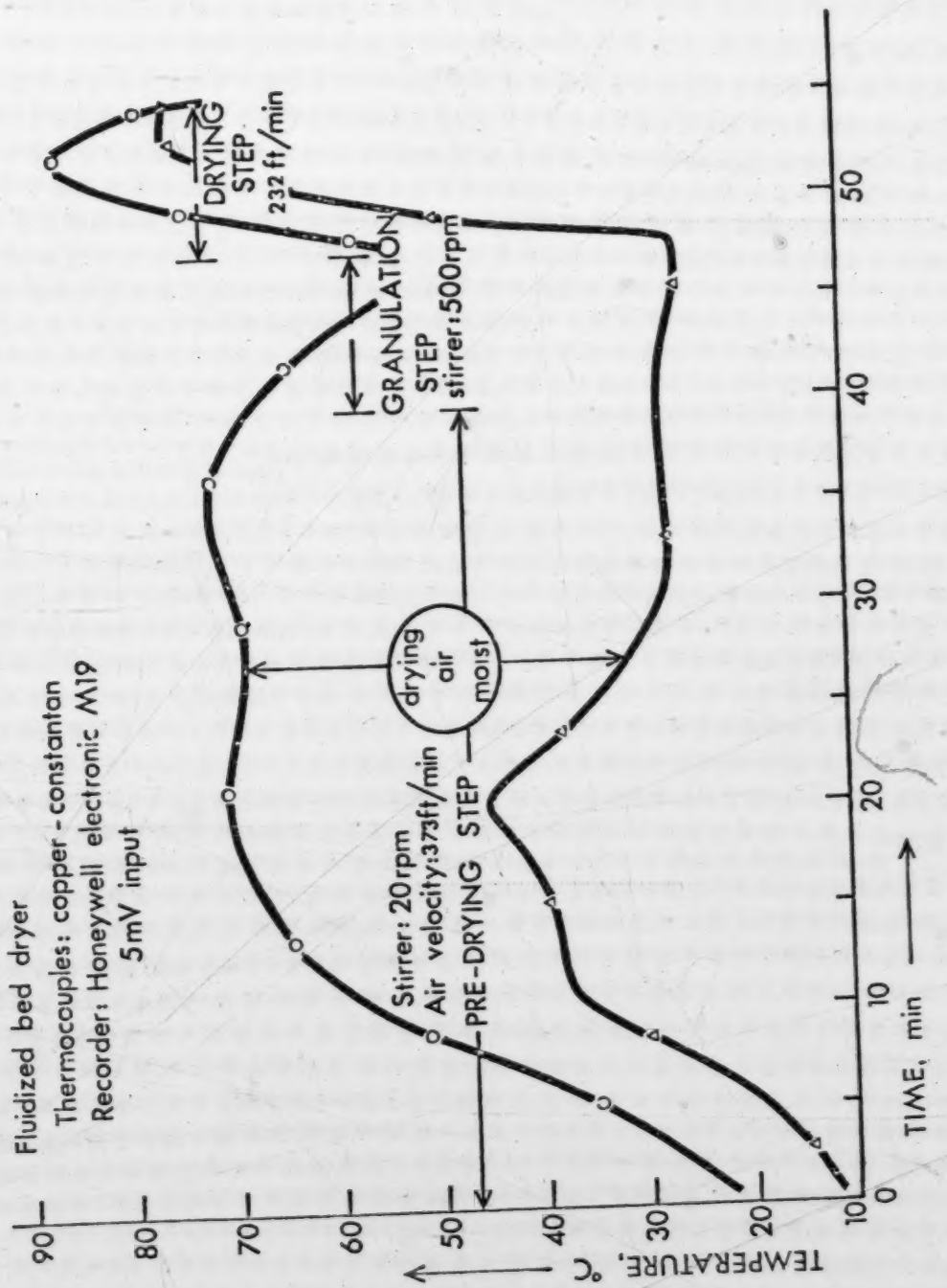


Figure 9. The temperatures of drying air and of potato mash vs time during pre-drying, granulation, and final drying steps of the freeze-thaw process.

Table 6. Pyrazine losses during pre-drying, granulation, and final drying steps of potato granule processing by the freeze-thaw technique.

Pyrazine	Processing step	Moisture content, %*			Pyrazine loss, %		
		1**	2	3	1	2	3
Methyl-	Pre-drying	57.5	50.3	53.9	67.0	64.4	65.7
	Granulation	35.5	24.1	29.8	95.1	95.9	95.5
	Final drying	6.3	6.5	6.4	99.9	99.9	99.9
2-Methyl -3-ethyl	Pre-drying	41.5	39.8	40.6	82.3	87.7	84.9
	Granulation	22.5	20.1	21.3	99.4	99.2	99.3
	Final drying	7.9	6.6	7.2	99.9	99.9	99.9
2,3,5-Tri -methyl	Pre-drying	51.8	44.1	46.8	81.0	80.0	80.5
	Granulation	36.0	25.6	30.8	97.9	99.7	98.8
	Final drying	6.4	6.8	6.6	99.9	99.9	99.9
2-Methox y-3-eth- yl	Pre-drying	49.6	38.1	43.9	74.6	87.7	81.1
	Granulation	24.2	22.1	22.6	96.5	99.1	97.8
	Final drying	7.4	6.6	7.0	99.9	99.9	99.9

*Moisture determination: 5g sample dried at 70°C for 24 hr, vacuum oven.

**Pilot scale batch runs.

cess appeared to be dependent only on the amount of moisture removed. Thus, in pre-drying, each percent drop in moisture content was accompanied by an average 1.445 mg percent loss of pyrazines, irrespective of their boiling points or structures. Also, non-selective retention of pyrazines was found during granulation, in which each percent drop in moisture produced a pyrazine loss of 1.16 mg percent. The only exception was 2-methyl-3-ethylpyrazine, for which the loss was 0.74 mg%. In final drying, where the moisture removed was 14 to 24.2%, the loss was 0.05 mg percent for trimethyl- and 2-methyl-3-ethylpyrazine, and 0.12-0.19 mg percent for other pyrazines.

Thus, only trace amounts (0.01 to 0.04% of the initial amount added) of pyrazines were detected in the end-product. Of interest was the finding that 2-methoxy-3-ethylpyrazine, with the lowest boiling point among the compounds tested, was retained by 0.04% in all runs.

The data in Table 6 were corrected for the presence of natural pyrazines in the mash and for pyrazines formed during dehydration. Koehler and Odell (1970), in work on pyrazine formation in a sugar-amine model system, found that measurable pyrazine formation started to occur at 100°C and increased rapidly up to 150°C, after which the pyrazine yields declined. Thus, one would expect significantly high levels of naturally occurring pyrazines in potatoes steam cooked at temperatures of 100°C or higher, with the amount decreasing during vaporization resulting at the mashing step. Also, heat artifact formation could be expected at temperatures approaching 100°C in the dehydration process. Findings in this study illustrate these expectations. For example, the moist mash, with an initial moisture content of 79.36%, gave a cathodic wave with $E_{1/2}$ 0.788V. Its height, when compared to that of pure meth-

ylpyrazine, corresponded to a level of 2.9 parts per 10^8. This represented an approximate ten-fold reduction in pyrazine concentration from the level found in steam cooked potato (3.8 parts per 10^7, calculated from cathodic wave with $E_{1/2}$ 0.788V). When the mash was pre-dried, granulated and dried to a moisture content of 6-7%, the pyrazine level found in mashed potato increased to 1.7 parts per 10^7, and much higher gains were recorded when final drying was performed at 85°C.

b. Add-back and drum-dry processes

Extracts from potato flakes and granules, and from powdered imitation potato flavor, had $E_{1/2}$'s ranging from -0.77 to -1.24V (Table 7). As shown in Table 4, this range was adequately covered by the reduction patterns of the four pyrazines studied. Since many of the pyrazines displayed 2, 3 or 4 reduction waves ranging from -0.781 to -1.441V, it was probable that the diffusion currents for the commercial potato samples arose either from the multi-stage reduction of one major pyrazine, or from the presence of two or more pyrazines exhibiting a single or multi-step reduction profile.

The possibility of the presence of other polarographically active compounds in commercially processed granules and flakes, which would interfere with the pyrazine waves, was also investigated. The most probable interference was expected from aldehydes produced in rancidity development during storage. Hexanal, the major off-flavor in flakes and granules, had an $E_{1/2}$ within the range of peak 3 (from Table 7). The major hexanal peak was at -1.12V, while an additional peak - shouldered by a small peak shifted 50 mV positively - was found at -0.76V. These peaks appeared not to be dependent on concentration but, rather, on non-diffusion controlled processes and/or the slight water

Table 7. Pyrazines in ppm in some commercially processed dehydrated potatoes. The results were based on individual peak heights recorded by differential pulse polarography.

Sample	Peak	1	2	3
		Half-wave potentials (-V)		
		0.77-0.80	0.91-0.94	1.09-1.24
Add-back granules*		0.17**	-	0.19
Freeze-thaw granules*		0.19	0.15	0.35
Flakes, brand A		0.38	-	2.48
Flakes, brand B		0.15	-	0.17
Imitation potato flavor, powder		2.29	1.64	3.65

*Freshly prepared.

**All values an average of three determinations which did not vary by more than ±5%.

solubility of hexanal. In order to completely avoid hexanal interference, only freshly processed samples were analyzed, though it was found that hexanal was removed with dichloromethane and was not transferred to the aqueous phase during the organic solvent evaporation step.

Most alkylated pyrazines have an $E_{1/2}$ of -0.75 to -0.80V. Hence, the occurrence of this wave in all commercial samples analyzed strongly suggested their presence in processed potatoes. The wave at $E_{1/2}$ -0.91 to -0.94V in freeze-thaw granules and imitation flavor powder, which coincided with the major wave of 2-methoxy-3-alkylated pyrazine, also suggested the presence of alkoxy compounds. Finally, in all samples analyzed, the occurrence of a third diffusion current at $E_{1/2}$ -1.09 to -1.24V, which consisted either of one wave or a wave shouldered by an additional one or two waves, merely reflected the behavior of both alkylated and alkoxy pyrazines at more negative potentials.

Quantitative results shown in Table 7 indicated that granules processed by a freeze-thaw technique had, as would be expected, a better overall flavor quality than granules of the add-back process, since both alkylated and alkoxy pyrazines were present. This suggestion was in agreement with earlier results of a sensory panel evaluation (Ooraikul, 1973). The absence of an alkoxy pyrazine wave in add-back granules was probably due to the steam evaporation being repeated several times in the process.

The results for potato flakes appeared to reflect the degree of heat application, which is high in the drum-drying step. Alkoxy derivatives were not detected, while the levels of alkylated pyrazines differed for the two brands analyzed. Brand B, which contained the imitation flavor, had a lower level of these compounds, which suggested that

another class of compounds was used for flavor enhancement. On the other hand, commercial imitation flavor, when compared to freeze-thaw granules, contained 12.7 and 11.0 times the amount of alkylated and alkoxy pyrazines, respectively.

2. Potato cellular components

As mentioned earlier for potato granule processing, the highest temperatures of the drying air at the drier base and at the exhaust pipe were 86° and 60°C, respectively (Figure 9). The average temperature of the pure component matrices during processing was 35°C. A significant change in processing procedure from that employed for potato granule production was necessitated, however, due to the low initial moisture contents of the pyrazine enriched matrices. In order to facilitate fluidization of these particles, which were much more smaller and adherent than potato cells, the amount of 1% pyrazine solution added was low. The initial moisture levels of moist starch, amylose, amylopectin, pectin and cellulose were 42.21, 60.63, 60.22, 39.99 and 58.78%, respectively. These values were much lower than the water content of mashed potato, but the moist mixes had similar consistencies to that of mashed potatoes. Thus, it was necessary to reduce times of pre-drying, granulation and final drying. These steps in the process were arbitrarily designated according to the time involved for each operation. Hence, the curves in Figure 9 remained essentially the same, with the exception of the length of the entire dehydration process.

Losses of methyl-, 2-methyl-3-ethyl-, 2,3,5-trimethyl- and 2-methoxy-3-ethylpyrazine during freeze-thaw dehydration of moistened potato starch, amylose, and amylopectin, pectin and microcrystalline cellulose, enriched with these pyrazines, are presented in Tables 8 to

12. All of the matrices tested showed the inability, as did potato granules, to retain any pyrazines, with the loss of these compounds being at least 99.9% after the final drying step. In addition, the major pyrazine losses were again observed during the pre-drying step, with values ranging from 47.4 (loss of methylpyrazine from microcrystalline cellulose) to 89.6% (loss of 2-methyl-3-ethylpyrazine from cellulose). Although many of the losses which occurred during pre-drying were considerably lower than those experienced during production of potato granules, the values were not a true comparison of retaining abilities between potatoes and potato constituents, since processing parameters varied substantially. During granulation, additional losses were incurred, which amounted to anywhere from 2 to 40%. Final drying brought about the completion of the near total loss of pyrazines which occurred during the dehydration process.

There did not appear to be any significant differences in pyrazine retention between the various component matrices. Each macromolecular constituent of the potato cell displayed little, if any, affinity for pyrazines regardless of the nature of the ring substitution on the pyrazine molecules. The extent of pyrazine removal again appeared to be dependent upon the amount of moisture removed. This finding, which is identical to that reported for pyrazine removal from mashed potatoes, suggests that, in regards to the location of pyrazines within potato cells or potato component mixes, the pyrazines are dissolved in the water (inter- and/or intracellular or particulate) associated with potato cells or cellular component particles. Thus, as the moisture is removed, pyrazines are also evaporated, even at low limiting moisture contents.

Table 8. Pyrazine losses during pre-drying, granulation, and final drying steps of pure potato starch dehydration by the freeze-thaw technique.

Pyrazine	Processing step	Moisture content, %*			Pyrazine loss, %		
		1**	2	3	1	2	3
Methyl-	Pre-drying	30.3	29.8	28.9	60.3	62.2	67.0
	Granulation	16.6	14.9	18.2	87.4	93.0	83.7
	Final drying	7.0	6.3	6.7	99.9	99.9	99.9
2-Methyl -3-ethyl	Pre-drying	26.7	33.9	31.8	71.1	66.6	69.9
	Granulation	12.0	19.1	17.7	95.5	88.8	88.9
	Final drying	6.5	6.5	6.5	99.9	99.9	99.9
2,3,5-Tri- methyl	Pre-drying	34.4	33.6	33.8	69.9	69.2	68.7
	Granulation	15.9	16.5	15.6	92.3	91.9	95.2
	Final drying	5.3	6.9	7.0	99.9	99.9	99.9
2-Methoxy- 3-ethyl	Pre-drying	30.1	33.2	27.7	66.8	66.7	70.5
	Granulation	14.0	14.4	10.0	93.4	94.3	97.9
	Final drying	6.5	6.9	5.9	99.9	99.9	99.9

*Moisture determination: 5g sample dried at 70°C for 24 hr, vacuum oven.

**Pilot scale batch runs.

Table 9. Pyrazine losses during pre-drying, granulation, and final drying steps of pure potato amylose dehydration by the freeze-thaw technique.

Pyrazine	Processing step	Moisture content, %*			Pyrazine loss, %		
		1**	2	3	1	2	3
Methyl-	Pre-drying	45.9	48.2	41.7	70.4	63.0	76.8
	Granulation	29.9	29.3	26.1	87.9	87.8	93.0
	Final drying	7.2	7.1	7.3	99.9	99.9	99.9
2-Methyl -3-ethyl	Pre-drying	38.9	40.1	35.2	75.5	73.7	77.6
	Granulation	19.2	22.2	18.0	95.9	94.8	95.9
	Final drying	7.4	6.7	6.7	99.9	99.9	99.9
2,3,5-Tri- methyl	Pre-drying	36.3	33.2	30.1	73.0	75.0	80.1
	Granulation	15.7	22.9	17.7	97.6	94.5	95.4
	Final drying	6.5	6.3	7.1	99.9	99.9	99.9
2-Methoxy- 3-ethyl	Pre-drying	39.3	40.2	44.4	74.7	73.7	68.9
	Granulation	12.7	13.1	18.8	97.9	97.5	92.5
	Final drying	6.8	6.9	6.8	99.9	99.9	99.9

*Moisture determination: 5g sample dried at 70°C for 24 hr, vacuum oven.

**Pilot scale batch runs.

Table 10. Pyrazine losses during pre-drying, granulation, and final drying steps of pure potato amylopectin dehydration by the freeze-thaw technique.

Pyrazine	Processing step	Moisture content, %*			Pyrazine loss, %		
		1**	2	3	1	2	3
Methyl-	Pre-drying	40.1	40.9	37.3	67.7	67.9	69.7
	Granulation	13.3	22.4	22.5	96.6	83.7	83.5
	Final drying	6.1	6.1	5.7	99.9	99.9	99.9
2-Methyl -3-ethyl	Pre-drying	43.9	40.1	42.8	68.3	69.3	69.2
	Granulation	26.9	24.3	23.5	78.9	83.4	82.9
	Final drying	7.0	6.8	6.7	99.9	99.9	99.9
2,3,5-Tri- methyl	Pre-drying	45.0	39.8	41.6	62.7	70.2	65.6
	Granulation	22.5	18.9	18.5	81.9	84.3	84.9
	Final drying	7.0	7.2	6.9	99.9	99.9	99.9
2-Methoxy- 3-ethyl	Pre-drying	40.3	39.7	38.8	67.5	68.9	69.9
	Granulation	25.4	20.1	27.7	89.9	90.3	84.5
	Final drying	6.4	6.3	6.9	99.9	99.9	99.9

*Moisture determination: 5g sample dried at 70°C for 24 hr, vacuum oven.

**Pilot scale batch runs.

Table 11. Pyrazine losses during pre-drying, granulation, and final drying steps of pure pectin dehydration by the freeze-thaw technique.

Pyrazine	Processing step	Moisture content, %*			Pyrazine loss, %		
		1**	2	3	1	2	3
Methyl-	Pre-drying	30.1	32.1	33.2	59.5	57.8	55.8
	Granulation	10.9	9.8	12.3	98.9	97.9	96.3
	Final drying	5.1	5.0	5.9	99.9	99.9	99.9
2-Methyl -3-ethyl	Pre-drying	29.7	24.6	29.6	60.1	66.6	60.4
	Granulation	8.1	12.9	15.2	98.9	95.1	93.0
	Final drying	5.0	5.5	6.0	99.9	99.9	99.9
2,3,5-Tri- methyl	Pre-drying	23.1	33.7	26.6	63.7	50.2	59.8
	Granulation	15.1	16.2	11.1	93.1	93.0	95.2
	Final drying	6.3	5.8	5.9	99.9	99.9	99.9
2-Methoxy- 3-ethyl	Pre-drying	28.8	27.0	26.6	79.9	80.2	85.9
	Granulation	14.2	18.2	17.9	95.3	94.9	94.3
	Final drying	6.3	6.1	6.6	99.9	99.9	99.9

*Moisture determination: 5g sample dried at 70°C for 24 hr, vacuum oven.

**Pilot scale batch runs.

Table 12. Pyrazine losses during pre-drying, granulation, and final drying steps of pure cellulose dehydration by the freeze-thaw technique.

Pyrazine	Processing step	Moisture content, %*			Pyrazine loss, %		
		1**	2	3	1	2	3
Methyl-	Pre-drying	50.4	50.3	49.8	50.0	50.0	47.4
	Granulation	41.6	42.8	40.0	79.2	80.9	78.9
	Final drying	5.4	6.6	6.0	99.9	99.9	99.9
2-Methyl -3-ethyl	Pre-drying	24.5	24.2	25.6	88.8	89.6	82.2
	Granulation	13.1	14.2	19.0	95.4	92.0	90.0
	Final drying	5.1	5.5	5.5	99.9	99.9	99.9
2,3,5-Tri- methyl	Pre-drying	37.7	43.9	24.6	72.0	59.3	85.7
	Granulation	22.0	19.0	25.6	90.3	92.2	88.4
	Final drying	5.5	7.0	6.3	99.9	99.9	99.9
2-Methoxy- 3-ethyl	Pre-drying	38.4	43.3	40.1	74.4	63.7	65.7
	Granulation	20.0	21.0	23.3	93.7	90.0	90.1
	Final drying	6.1	5.9	6.8	99.9	99.9	99.9

*Moisture determination: 5g sample dried at 70°C for 24 hr, vacuum oven.

**Pilot scale batch runs.

Comparisons of any retention discrepancies involved as a result of volatility differences between the four pyrazines tested was meaningless because of the difficulty experienced in a fine process control, as times of pre-drying, granulation and final drying had to be substantially reduced due to low initial moisture contents. Any reliable comparisons would not be significant considering the final retention values.

A parallel study with potato granules involving scanning electron microscopy (Moledina *et al.*, 1977) showed that most freeze-thaw granules were single intact cells, with randomly arranged cellulose fibres and an approximately equal amount of pectin material, within the surrounding cell walls. This would suggest that pyrazine retention, if dependent on cell wall affinity, would also be influenced to a great extent by cellulose and pectin *per se*. In addition, other intracellular components such as starch, amylose, amylopectin and protein have displayed little or no ability to retain pyrazines, thus further emphasizing the relative importance of cellulose and pectin as possible factors in flavor volatile retention. The results reported for the effect of freeze-thaw processing on pyrazine retention by these two components support the fact that retention is virtually non-existent during production of dehydrated potato granules. Neither cellulose nor pectin were able to retain pyrazines even during the rather short process utilized in this study.

The assumed lack of cell wall impermeability in the potato granule, which would allow the small pyrazine molecules easy access to the cell exterior, can be illustrated by some recent scanning electron microscopy studies that followed granule formation during pre-dry-

ing, granulation and final drying (Moledina *et al.*, 1977). An electron micrograph of the outside surface of final dried potato granules, magnified 2,000 times (Figure 10-a), showed the occurrence of small, rectangular particles on the outer granule surface. These crystal-like structures were approximately $0.5\mu^3$ in size and were also found on the granule surface mid-way through the granulation step (Figure 10-b) and immediately following the pre-drying step (Figure 10-c). These particles were found to be either KCl or phosphate salt crystals which were formed as a result of leakage through the cell wall. Hence, the cell wall of the potato granule was shown to be completely permeable to the movement of small molecules throughout the dehydration steps, thus giving support to the near complete loss of pyrazine flavorings during the freeze-thaw dehydration process.

B. Pyrazine retention in model systems

Micro Kjeldahl analysis of fresh tissue from Russet Burbank tubers showed the protein content to be 8.12% on a dry basis (a factor of 7.25 was used for conversion of nitrogen to protein). Low yields of potato protein were obtained from the heat coagulation and salt extraction of juice protein. For example, a total of 3.2g protein was obtained from an initial 750g of fresh tissue, which represented a protein content of 1.71% and, thus, a yield of only 21.06%.

Approximately 75% of the total protein from fresh potato tissue was found in the juice. The remaining potato cake had a protein content of 2.47%, with a moisture content of 30.44%. Solvent extraction of this cake revealed that, after three extractions (3:1 v/w) with water, 10% NaCl, 70% ethanol and phenol:acetic acid:water (2:1:1, v/v/v), the percentages of the initial cake protein remaining in the cake were

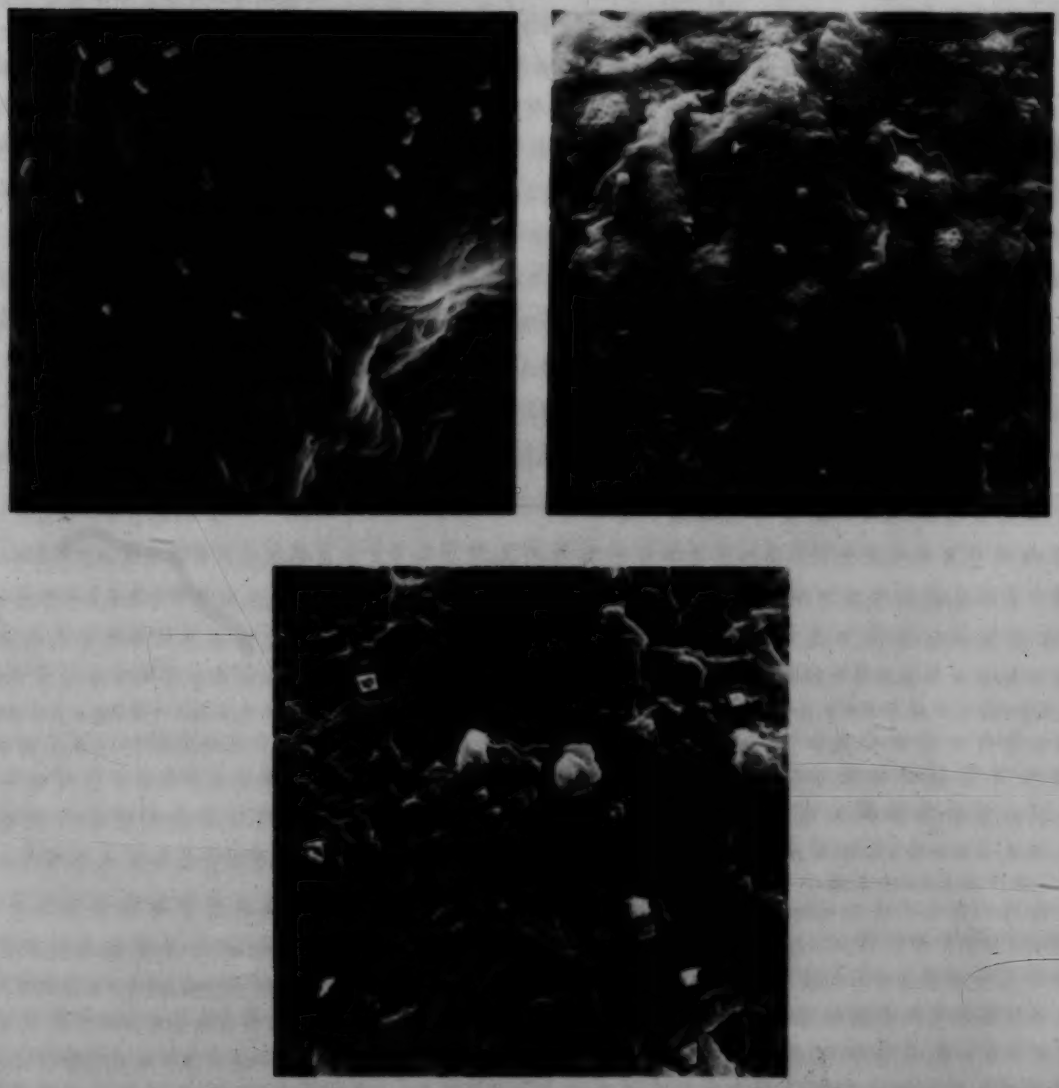


Figure 10. Scanning electron micrographs (magnification 2,000 X) of crystal formation on outer granular surfaces during production of dehydrated potato granules by a freeze-thaw dehydration process.

a. Final dried granules

b. Granulated potato

c. Pre-dried potato

43.99, 75.75, 40.51 and 6.69, respectively. This indicated that the potato cake did not contain one unique protein fraction, since water, salt and alcohol soluble proteins were extracted (the phenolic solvent was recommended by Desborough and Weiser, 1972, for total protein extraction). Thus, the process of juice expulsion probably reduced the total protein content of the cake rather than selectively extracting particular protein fractions. The juice protein was therefore considered to be an adequately representative sample of the total protein composition, and the cake was discarded during protein extraction procedures.

A prerequisite for reliable results concerning the elution speed of a compound through a column matrix is the ability of the molecules of that compound to maintain a very narrow band during their travel through the column. Each retention value in this study is based on the elution profile of the first twenty fractions from the column. Examples of these profiles are shown in Figure 11. All of these retention curves were sharp peaks, proving that the pyrazines travelled through the column in a very narrow band. The results for retention of methyl-, 2-methyl-3-ethyl-, 2,3,5-trimethyl- and 2-methoxy-3-ethylpyrazine, in model systems of packed columns containing add-back dehydrated potato granules and pure macromolecular components of the potato, are presented in Table 13. In general, retention of pyrazines was very low, with values ranging from 0.1 to 64.5% retention after elution with only 40 ml of water-saturated dichloromethane (pH 5.35). For practical purposes, the eluent volume was arbitrarily chosen; further elution would reveal residual pyrazines on the column, however, these remaining compounds would probably be more of a reflection of the relatively

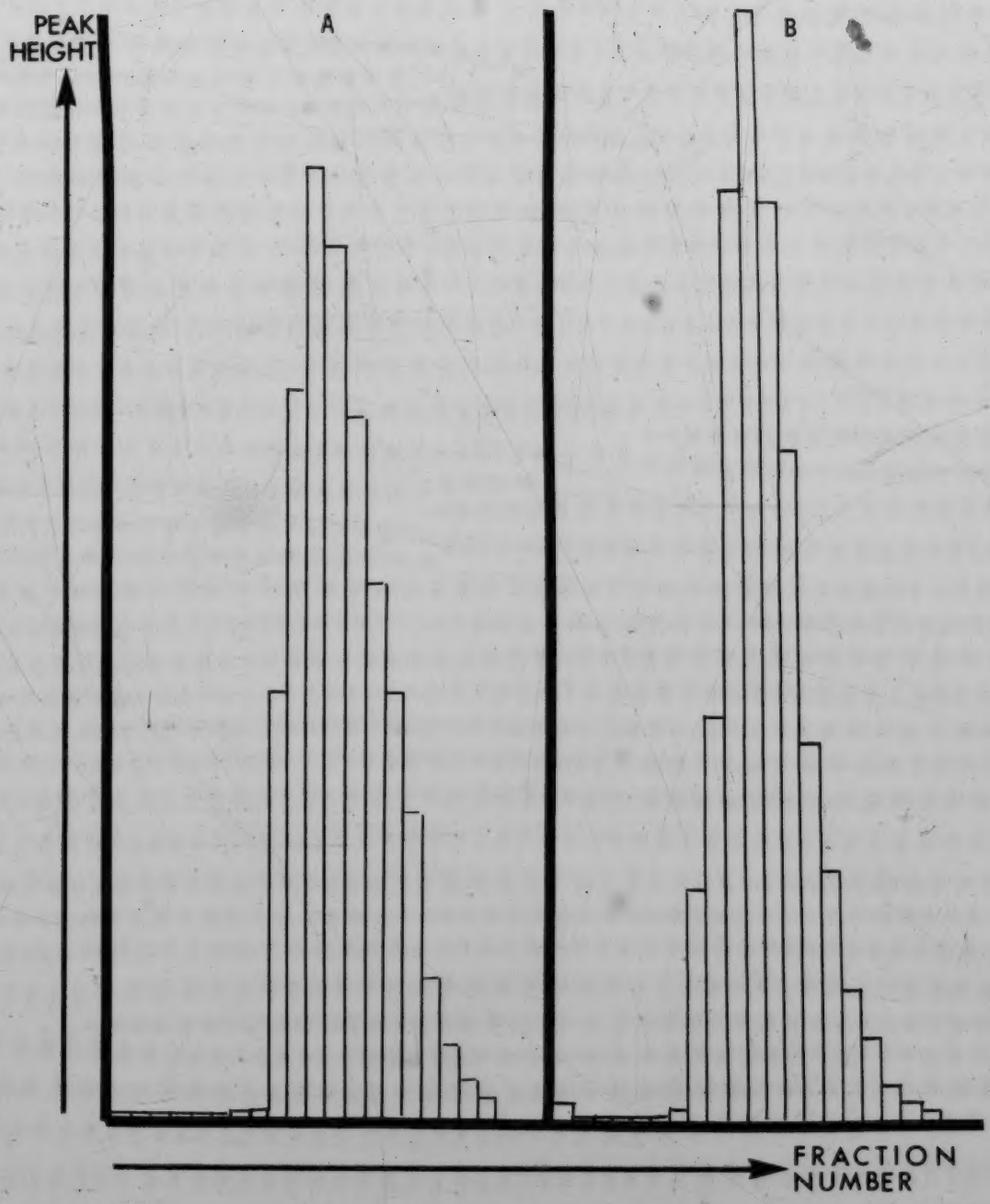


Figure 11. Elution profiles of methylpyrazine on a column of pure potato protein eluted with water-saturated dichloromethane; A= 10 ppm methylpyrazine, B= 1 ppm methylpyrazine.

Table 13. Pyrazine retention during dichloromethane elution of columns containing dehydrated potato granules and potato cell component matrices.

Matrix	Retention, %*							
	Methylpyrazine, ppm		2-Methyl-3-ethylpyrazine, ppm		2,3,5-Trimethylpyrazine, ppm		2-Methoxy-3-ethylpyrazine, ppm	
	1	10	1	10	1	10	1	10
Starch	4.2	2.8	25.8	14.0	14.1	11.5	16.8	0.1
Amylose	0.1	0.1	20.9	12.0	15.1	15.8	27.0	0.1
Amylopectin	3.3	4.0	6.0	7.8	18.9	14.9	20.2	3.6
Pectin	0.1	5.6	16.3	10.7	0.1	7.4	4.9	4.7
Cellulose	38.1	35.6	28.7	26.9	27.7	18.5	64.5	27.9
Protein	12.1	24.7	25.3	19.0	20.1	16.2	14.6	5.9
Add-back potato granules	4.7	4.3	15.6	14.2	17.9	7.2	22.2	0.1

*Based on comparison of total peak heights for 1st 20 2ml fractions to peak height of total pyrazine applied to the column.

small number of fractions collected rather than true values of retention. Considerable discrepancies in retention values for each pyrazine at different concentrations were observed in several cases, and these were probably a result of many factors including slight variations in pyrazine application, eluent flow rate and fraction volume collected, as well as the chemical and physical changes induced in the column.

There did not appear to be any significant trend in retention potential among the four pyrazines tested; that is, no single pyrazine was consistently retained more than the other pyrazines. None of the individual matrices revealed the capacity to hold on to the pyrazines strongly. Cellulose showed the highest overall affinity for the pyrazines, with an average retention value, for all four compounds, of 32.7%. This value was most likely a reflection of the extremely large surface area of the cellulose crystals (which were less than 100μ in size), and, thus, the possibility for hydrogen bonding and surface adsorption was much greater than for the other components. Potato protein displayed the second highest retention ability, with an average value of 17.2%. This was not unexpected since the pyrazines, which are basic in nature, can form salt linkages with the acidic amino acids in the protein chain. The extent of this type of bonding, however, would depend on the pH as well as the isoelectric points of the various potato protein fractions. The remaining potato carbohydrate components - starch, amylose, amylopectin and pectin - as well as the add-back dehydrated granules, gave much lower average retention values than cellulose and protein. This was also expected, considering the structures of each, which are lower in hydrogen bonding sites and

acidic groups. It should be noted, however, that retention comparisons between matrices showing such low values (0.1-25%, on the average) was probably not entirely meaningful on a chemical basis, since these low figures may have merely indicated the arbitrary column parameters imposed in the model system studies. Moreover, the significant finding within this study suggested that strong, covalent-type bonding between pyrazines and potato granules and potato cell constituents is extremely unlikely, even at limiting moisture contents.

V. DISCUSSION

This study revealed that freshly mashed potato had a total pyrazine level of 2.9 parts per 10^8. After the mash was processed into granules by a freeze-thaw dehydration technique, differential pulse polarography indicated a level of 1.7 parts per 10^7, though part of the flavor of freshly mashed potato was lost during processing. This represented an approximate ten-fold increase in the total pyrazine content. A parallel study showed that granule dehydration brought about an interaction of amino acids and sugars which inflated the polarographic waves (Steele *et al.*, 1976). The cathodic wave of these amino acid-sugar Maillard reaction products ($E_{1/2}$ near -0.75V) coincided with that of pure alkylated pyrazines. In addition to this common mechanism for the formation of pyrazines and pyrazine-like compounds, a variety of basic, pyrazine-like compounds have been shown to be formed during pyrolysis of oxy-α-amino acids (Kato *et al.*, 1970) and in model systems of carbohydrates and aqueous ammonia (van Praag *et al.*, 1968). Although the latter conditions do not reasonably approximate actual granule production circumstances under which these compounds are formed, it can be seen that the possibility exists for the formation of numerous basic compounds, both volatile and non-volatile, which may be polarographically active. Therefore, the results given here do not solely represent the potent potato flavoring compounds found initially in the tuber, but, rather, all pyrazines, including flavorless, non-volatile, pyrazine-like derivatives synthesized before or during dehydration steps. Also, since these carbohydrate-amino acid interactions are much more predominant at higher temperatures (considerable amounts of pyrazines may be formed at temperatures of 100°C or higher), final dehydration

carried out at 85°C would favor the production of these compounds. The formation of these derivatives during the drying operations accounted for the apparent discrepancy found between flavor loss and pyrazine gain, as was detected in both add-back and freeze-thaw granules. Thus, quantitative evaluations regarding pyrazine levels in potatoes before, during and subsequent to processing treatments must take into consideration the balance between pyrazine losses due to operational circumstances and pyrazine gains resulting from heat artifact formation. Although an actual increase in the total natural pyrazine content of mashed potato was substantiated, both levels, though easily detectable, were exceedingly low from a flavor standpoint and thus, the improvement of the flavor quality of potato granules, by using synthetic flavors, appeared as desirable.

From a consumer standpoint, one of the important determinants in purchasing convenience food items such as instant dehydrated mashed potatoes on a continual basis, aside from the convenience factor, is the flavor quality. The types of dehydrated potato products currently available on domestic retail shelves seem to be totally limited to the flake form. Dehydrated potato granules have not as yet found their way to retail markets to the extent that the flaked form has. Dehydrated potato flakes are being marketed with or without an imitation potato flavoring and, furthermore, the method employed for the incorporation of these synthetic flavors into the flakes is not known. Imitation potato flavorings are also commercially available, however, their composition is chemically not specified, and it is unknown as to whether the commercially produced artificial essences are utilized for flavor enrichment of commercially manufactured potato flakes. Despite

the lack of reliable information in this area, it is evident from Table 7 that the flavor quality of two brands of the flakes and one powdered imitation flavor, as influenced quantitatively and qualitatively by total pyrazine levels and the presence of both alkylated and alkoxy pyrazines, is somewhat questionable. Hence, flavor quality improvement of potato flakes and imitation flavoring with pure, synthetic pyrazines, as suggested for granules, again seems to be justifiable.

Several patents have been developed for flavor enhancement of processed potatoes with synthetic pyrazines (Flament, 1974; Guadagni *et al.*, 1973; Mookerjee *et al.*, 1973; Nakel and Dirks, 1971; Roberts, 1968), and other foods (Maga and Sizer, 1973). However, the feasibility of these patents for implementation in potato granule processing was not well elaborated, that is, specific recommendations were not given in regards to the exact procedures to be utilized in flavor augmentation at a particular stage in the dehydration process. This study demonstrated that flavor enrichment with pyrazines, with their incorporation at the mashing step of granule processing to ensure uniform distribution of the flavor, was not suitable, since flavor loss of at least 99.9% occurred in subsequent dehydration steps. This loss was not affected by the structures or boiling points of the volatile pyrazines. As a result of these retention studies, those patents suggesting pyrazine addition to potatoes prior to drying operations and other processing treatments that would cause extensive losses of these compounds, would be disqualified as being totally justifiable. Hence, the only practical procedure for flavor improvement appears to be the addition of flavors to the dehydrated end-products, analogous to the method now being employed for enrichment of granules with Vitamin C

(Steele *et al.*, 1976).

Due to the volatile nature of the pyrazine flavoring compounds, the only practical method for pyrazine enrichment of processed potatoes would be one in which the pyrazines are incorporated as immobilized compounds and, thus, upon reconstitution with warm water, the flavor required to match that of freshly mashed potatoes would be released. A notable United States patent (Guadagni *et al.*, 1973), in which processed potatoes are enriched with immobilized pyrazines, recommended the addition of pyrazines in the form of a hydrochloric acid salt, which would, presumably, release the pyrazines once the acid is neutralized by the buffering capacity of reconstituted granules. What appears to be a very promising method for flavor enhancement of processed potatoes with pyrazines is the impregnation of these compounds into macromolecular constituents of the potato granule, such as proteins and various carbohydrates. Initial investigations have been conducted regarding the interactions of flavor model compounds with model food components, although limited information was found concerning the formation of complexes between food components and pyrazines (Beyeler and Solms, 1974, studied the interaction of dimethylpyrazine with soy protein and bovine serum albumin). Results from these preliminary investigations are encouraging since a variety of flavor compounds had the ability to complex with several solid food matrices. For example, starch and its constituents can bind flavors in the form of inclusion complexes ("clathrates"), and the trapped compounds would be fully released following rehydration. Many flavors can bind to proteins through a hydrophobic process, and lipid-flavor aggregates can occur from simple partitioning effects. The elucidation of these effects with pyrazines

as the model flavors, however, is virtually non-existent and, hence, a great deal of work is required for a better understanding of the possibility of adding pyrazines to food macromolecules for purposes of flavor improvement of processed potato products.

In conclusion, this study showed that, by avoiding flavor addition before or during the dehydration steps of granule processing, all the suggestions given above offer a genuine improvement in the bland flavor of the granules presently supplied to consumer and institutional markets.

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